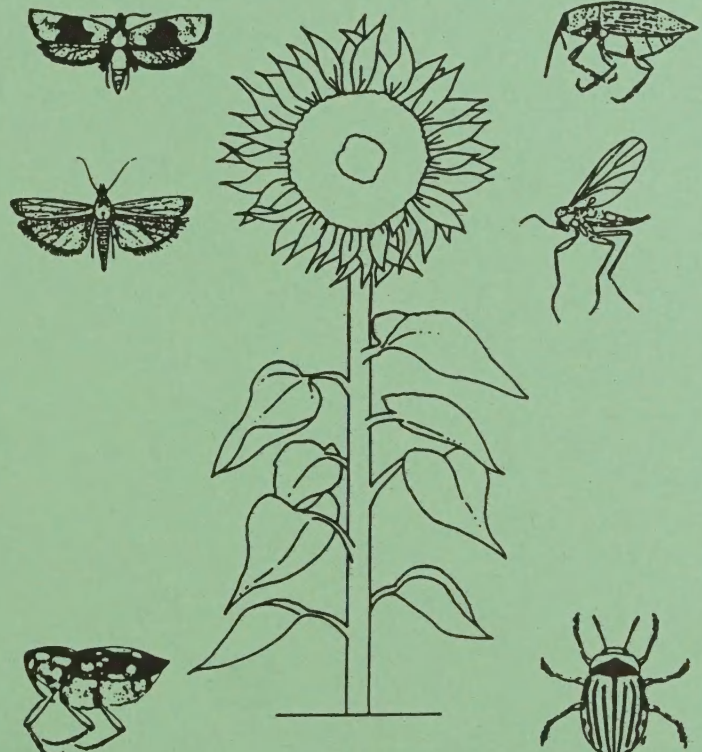
# *PROCEEDINGS*

# NINETH GREAT PLAINS SUNFLOWER INSECT WORKSHOP





**APRIL 18 - 19, 1996** **FARGO, NORTH DAKOTA**

# *PROCEEDINGS*

# NINETH GREAT PLAINS SUNFLOWER INSECT WORKSHOP

**April 18-19, 1996**

**USDA, ARS**
**Northern Crop Science Laboratory**
**Fargo, North Dakota**

**Workshop Chair & Proceedings Editor:**

**Larry D. Charlet**
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**The Great Plains Sunflower Insect Workshop was developed to foster communication, exchange information, and develop solutions to insect problems of common interest. This volume contains the program, a list of participants and the presentations from the 1996 Workshop.**

**The papers in these proceedings are not to be used without the expressed permission of the authors.**

**Copies of the proceedings are available from the Workshop Chair.**

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# 9th Great Plains Sunflower Insect Workshop
# 18-19 April 1996

*U. S. Department of Agriculture, Agricultural Research Service*
*Northern Crop Science Laboratory*
*Fargo, North Dakota*

## *Program & Schedule*

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8:00 - 8:30 **Registration**

8:30 - 8:35 **Welcome & Introduction** - **Larry Charlet**, *USDA, ARS, Northern Crop Science Laboratory, Fargo, ND*

8:35 - 10:00 **Presentations**

A possible role for cuticular lipids and the endocrine system in the regulation of inoculative freezing in the overwintering larva of the **red sunflower seed weevil**, *Smicronyx fulvus*. **Robert Rojas**, *USDA, ARS, Biosciences Research Laboratory, Fargo, ND*

Using DNA technology to identify sunflower pests and their parasitoids - **Richard Roehrdanz**, *USDA, ARS, Biosciences Research Laboratory, Fargo, ND*

Efficacy of different Bt formulations against the **banded sunflower moth**, *Cochylis hospes* - **Jawahar Jyoti**, *Department of Entomology, North Dakota State University, Fargo, ND*

Microsampling capitate glands of sunflower and its application for determining mechanisms of resistance for the **banded sunflower moth**, *Cochylis hospes* - **Mike Ellefson**, *Department of Entomology, North Dakota State University, Fargo, ND*

Effect of planting strategies on feeding by the **sunflower beetle**, *Zygogramma exclamationis*. **Craig Roseland & Teri Grosz**, *Department of Entomology, North Dakota State University, Fargo, ND*

**Discussion**

10:00 - 10:30 **Break and Refreshments**

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**Confirm** insecticide: chemistry and activity - **Gene Thilsted**, *Rohm & Haas Company, Houston, TX*

10:30 - 12:00 **Presentations (continued)**

The biology and management of the **sunflower stem weevil**, *Cylindrocopturus adspersus:* Past, present, and future - **Larry Charlet**, *USDA, ARS, Northern Crop Science Laboratory, Fargo, ND*

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12:00 - 1:30 **Lunch**

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The biology and management of the **sunflower seed weevils**, *Smicronyx fulvus* and *S. sordidus*. Past, present, and future - **Gary Brewer**, *Department of Entomology, North Dakota State University, Fargo, ND*

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The biology and management of the **sunflower moth**, *Homoeosoma electellum*. Past, present, and future - **Dick Wilson**, *USDA, ARS, Plant Introduction Station, Iowa State University, Ames, IA*

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*Friday, 19 April*

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The biology and management of the **sunflower beetle**, *Zygogramma exclamationis*. Past, present, and future - **Craig Roseland**, *Department of Entomology, North Dakota State University, Fargo, ND*

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| | |
|---|---|
| 10:00 - 11:00 | **Presentation** |
| | The biology and management of the **banded sunflower moth**, *Cochylis hospes*. Past, present, and future - **John Barker**, *USDA, ARS, Biosciences Research Laboratory, Fargo, ND* |
| | **Discussion** |
| 11:00 - 12:00 | **Integrated Pest Management of Sunflower - Discussion** |
| | Present & Future Prospects - where do we go from here? |
| | **Conclusion of Workshop** - comments, questions, future plans, etc. |

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# A Possible Role for Cuticular Lipids and the Endocrine System in the Regulation of Inoculative Freezing in the Overwintering Larva of the Red Sunflower Seed Weevil, *Smicronyx fulvus*

**Robert R. Rojas[1], Dennis R. Nelson[1], Roger A. Leopold[1], Samuel M. Newman[1] and Larry Charlet[2]**
**[1]Biosciences Research Laboratory and [2]Northern Crop Science Laboratory, USDA-ARS, State University Station, ND 58105, U.S.A.**

## ABSTRACT

In this report, we provide preliminary data that, in the mature larva of the red sunflower seed weevil, resistance to inoculative freezing may be due to physical changes occurring on the surface of the insect. Specifically, resistance to inoculative freezing is associated with a progressive increase in the larval surface lipids of the more hydrophobic components as seen in the hydrocarbons and triacylglycerols. Also, electron micrographs of the surface of a resistant larva showed what appeared to be a "waxy" covering over the spiracles. Finally, topical application of JH I to the larvae resulted in a reduction of resistance to inoculative freezing, while topical application of ecdysone resulted in an increase of resistance to inoculative freezing.

## INTRODUCTION

We have previously reported that the overwintering larva of the seed weevil, *Smicronyx fulvus*, developed a progressive resistance to inoculative freezing (1). **Inoculative freezing** is the internal freezing of an insect initiated by ice formation on the insect's surface as opposed to **nucleative freezing** in which freezing is initiated internally. Since the larva is not freeze-tolerant and spends the winter in a cold and moist environment it is crucial to its survival that it be able to resist this type of freezing.

The objective of this study was to characterize any changes on the larval surface during the acquisition of resistance to inoculative freezing. We

used surface lipid analysis and electron microscopy to detect gross structural changes on the larval surface. We also investigated the possibility of endocrine control of resistance to inoculative freezing by hormonal application to the larvae.

## MATERIALS AND METHODS

*Experimental Insects.* Larvae of the red sunflower seed weevil, *Smicronyx fulvus*, were obtained by collecting infested heads (capitula) of the sunflower plant from a test plot near Prosper, N.D. in late August to early September. Mature larvae were then collected in the laboratory as they emerged from the seeds while searching for overwintering sites. Larvae were placed into cylindrical plastic containers [8.5 x 4.7 (cm i.d.)] with four wire-mesh covered holes around the side and one in the bottom (2.5 cm dia.). The containers held soil and were fitted with plastic caps with a hole (2 cm dia.) cut into it and also covered with wire mesh. The containers were buried in a test field in Fargo, N.D. with the lids flush with the surface of the soil and were dug up on a monthly basis, brought into the laboratory, and the larvae were retrieved from the soil for analysis.

*Determination of Crystallization Temperature.* A single larva was placed in a large volume (60 µl capacity) stainless steel pan used for DSC analysis (Perkin Elmer, Norwalk, CT) either dry or in contact with 5 µl of distilled water and sealed. The sample was then cooled at a rate of 5°C/min in a Perkin Elmer DSC 7 to -40°C. A sample of 5 µl of distilled water was also run alone for comparison to the freezing behavior of the larva-contact water samples. The crystallization temperature ($T_c$) of the contact moisture was taken as the larger of the exothermic events and always preceded the $T_c$ of the larva. However, in some cases only one large exothermic peak with a slight shoulder was produced and in these cases freezing of the larva was taken as occurring at nearly the same temperature/time as the freezing of contact moisture.

*Electron Microscopy of Larval Surface.* Larvae selected for scanning electron microscopy were removed from the soil and washed in either distilled water or 0.1% Triton-X-100 in distilled water. Some larvae were then freeze dried as intact unfixed larvae. Other larvae were fixed by the

injection of glutaraldehyde in 0.2 M cacodylate buffer, pH 7.0. These larvae were then submerged in the fixative and left overnight. Fixed larvae were then rinsed in distilled water and treated with 1% aqueous osmium tetroxide for 2-3 hours, washed overnight in distilled water and then freeze dried. Dried samples were attached to standard aluminum sample stubs with double stick tape and silver paint adhesive (Ted Pella Inc. Redding, CA). The samples were "sputter coated" with a mixture of gold and palladium using a Humer V sputter coater to a thickness of 300-500 Å. The samples were examined with an AMR 1000 scanning electron microscope at accelerating voltages of either 10 or 20 kV. Images were recorded on Polaroid Type 55 P/N Film.

*Surface Lipid Analysis*. Seed weevil larvae were removed from the soil and freed of all debris. They were then placed in a champagne funnel and rinsed for 1.5 min with hexane (7-8 ml) followed by a rinse with $CHCl_3$ for spotting on TLC (thin-layer chromatography) plates (10x10 cm). The plates were developed in hexane:diethyl ether: formic acid (80:20:1), then air dried and sprayed with 5% $H_2SO_4$ in 95% ethanol. After allowing the ethanol to evaporate, the plates were heated for 10 min at 150°C after which the temperature was increased to 225°C and the plate was heated another 20 min to char the lipids on the plates.

The charred areas were quantified by scanning the image into a computer at 300 dpi (dots per inch) with a Hewlett-Packard ScanJet 4C using Adobe PhotoShop 3.0. The image file was then analyzed using Mocha 1.2 (Jandel Scientific). Analysis was conducted by measuring the Average Intensity and Number of Pixels of each of the charred lipid spots. Background values for each charred spot were determined by measuring the Average Intensity and Number of Pixels in an area close to and approximately the same size as the charred spot being measured. The average intensity of the background area was subtracted from the average intensity of the charred spot to give an adjusted average intensity. The adjusted average intensity was then multiplied by the number of pixels of the charred spot to give the total intensity.

The total intensity of the charred spot was then used either to determine the present composition of the lipid classes, or was converted to micrograms based on standard curves. The standard curves were obtained

by HPTLC of lipid standards from 0.5 to 6 µg: n-dotriacontane, 2-hexadecanol, henicosanyl acetate, arachidyl stearate, tetracosanoic acid, monostearin, distearin, tristearin, and cholesteryl palmitate.

*Hormonal Application.* Larvae kept in the laboratory at 20°C in soil were used for this part of the study. Larvae were removed from the soil and surfaced washed with distilled water to remove adhering soil particles. The hormones were applied topically. JH I was dissolved in acetone and 1 µl was applied which contained 1 µg of the hormone. Ecdysone was dissolved in ethanol and 1 µl was applied which contained 1 µg of the hormone. A control group received 1 µl of acetone without hormone. The treated insects were then placed on moist filter paper in plastic petri dishes, covered and kept at 20°C. The larvae were sampled for $T_c$ determination in the presence of contact moisture on the third and sixth day following treatment.

## RESULTS

A previous study of ours showed that the larval $T_c$ when determined on surface dry insects changed little throughout the overwintering period and into July (1). The mean $T_c$ remained low and constant from September to July at approx. -24°C (Fig. 1). The soil temperature profile for a given year indicates that a surface dry larva would avoid freezing (Fig. 2). However, we found that the presence of surface moisture on the larvae during cooling could dramatically raise the larval $T_c$ from -24°C when surface dry to -8°C when surface wet (Fig. 3) (2). This would put the larva at risk of freezing.

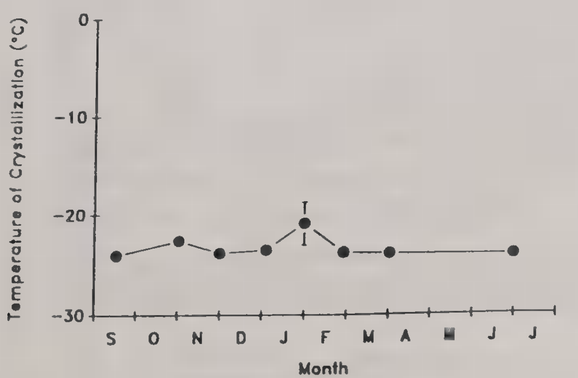


***Fig. 1.* Seasonal changes in larval whole-body temperature of crystallization ($T_c$) in the red sunflower seed weevil. Reprinted from (1).**

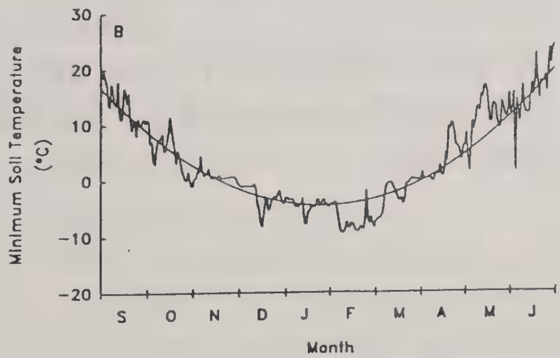


***Fig. 2.* The minimal daily soil temperatures recorded 5 cm below the soil surface with no snow cover. Reprinted from (1).**

However, when larvae were cooled in the presence of contact moisture the larval $T_c$ changed dramatically during the overwintering period (Fig. 4). Larvae collected from the soil in September were very susceptible to inoculative freezing. These larvae, in many cases, froze shortly after the contact moisture froze during cooling raising their $T_c$'s to ~-8°C (Fig. 4, top). By late October the larvae collected from the field containers begin to show some resistance to inoculative freezing (Fig. 4, middle). Then, in the middle of winter the larvae exhibited complete resistance to inoculative freezing (Fig 4, bottom).

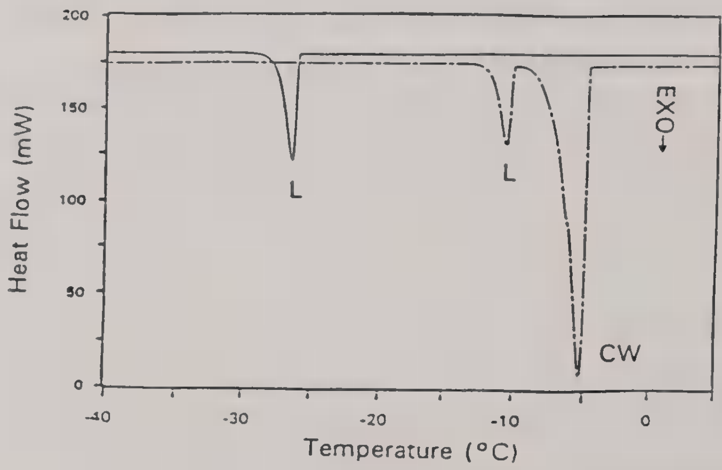


**_Fig. 3._ Representative DSC thermograms illustrating inoculative freezing by contact water (dashed line) and nucleative freezing (solid line) in a wetted and surface dry larva of the red sunflower seed weevil. L = $T_c$ of larva; CW = $T_c$ of contact water. Reprinted from (2).**

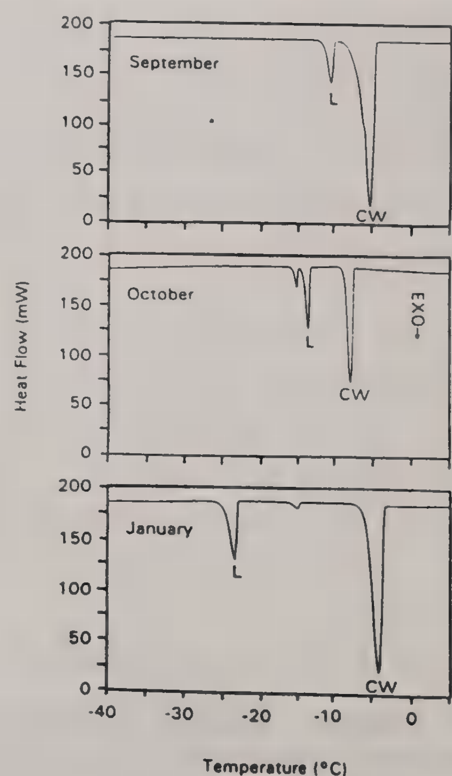


**_Fig. 4._ Representative DSC thermograms showing the seasonal resistance to inoculative freezing by contact water in mature larvae of the red sunflower seed weevil. Reprinted from (2).**

Any sites through which water is lost or gained from the insect are also potential sites for ice to grow through from the surface. One likely site for inoculative freezing to occur through are the respiratory openings (spiracles). Electron micrographs of the surface of resistant larvae revealed that the spiracles appear to be covered over with a "waxy" layer (Fig. 5). Respiration rates drop dramatically from the fall to mid-winter, the period when resistance to inoculative freezing increases.

Another, site for inoculative freezing to occur is through the cuticle itself. Epicuticular lipids are known to be important in controlling water loss in insects. We investigated the surface lipids during the seasonal progression of resistance to inoculative freezing. Three samples of the larvae were taken for surface lipid analysis by TLC. These samplings represent three different periods in diapause development associated with the development of resistance to inoculative freezing (1) early diapause and no resistance (2) middle diaqpause and intermediate resistance and (3) full diapause and complete resistance to inoculative freezing. TLC of the surface lipids of these


*Fig. 5.* **Electron micrographs of the surface of the mature larvae of the red sunflower seed weevil showing "waxy" coating over the spiracles.**

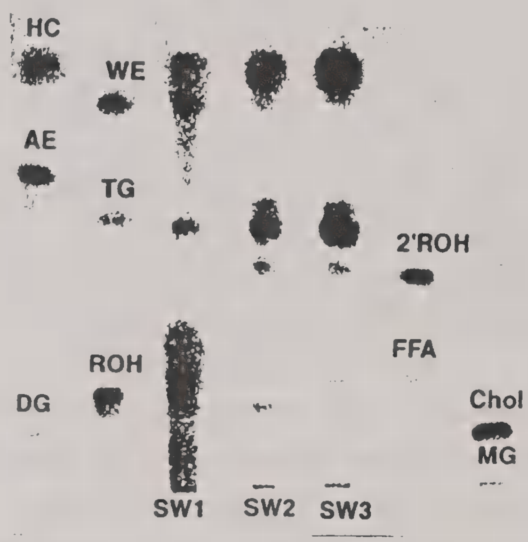


*Fig. 6.* **TLC plate of surface lipid extract from mature larvae of the red sunflower seed weevil. SW1 were collected in September, SW2 were collected in late October and SW3 were collected in February.**

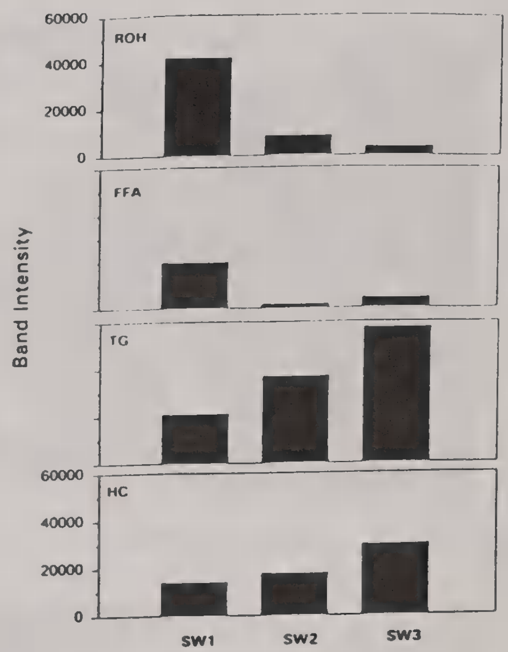


*Fig. 7.* **Band intensities of the different surface lipid components of the surface lipid extracts prepared from mature larvae of the red sunflower seed weevil. Band intensities were determined from the TLC plate shown in Fig. 6 .**

three groups of larvae revealed a change in the band intensities for certain lipid classes (Fig. 6 & 7). Specifically, resistance to inoculative freezing is associated with a progressive increase in the more hydrophobic lipid classes as seen in the hydrocarbons (HC) and triacylglycerols (TG).

Two insect hormones were topically applied to the larvae to see if it could alter their resistance to inoculative freezing. Ecdysone application tended to make the larvae more resistant to inoculative freezing over acetone treated controls (Fig. 8). Three days after ecdysone application the mean $T_c$ was -16.2 ± 3.9 vs. -13.0 ± 3.3°C and after 6 days the mean $T_c$ was -20.6 ± 2.0 vs. -15.6 ± 2.3°C. JH I application tended to have the opposite effect, making the larvae more resistant to inoculative freezing over acetone treated controls. Three days after JH I treatment the mean $T_c$ was -11.1 ± 1.2 vs. -13.0 ± 3.3°C and after six days -12.0 ± 1.3 vs. -15.6 ± 2.3°C.

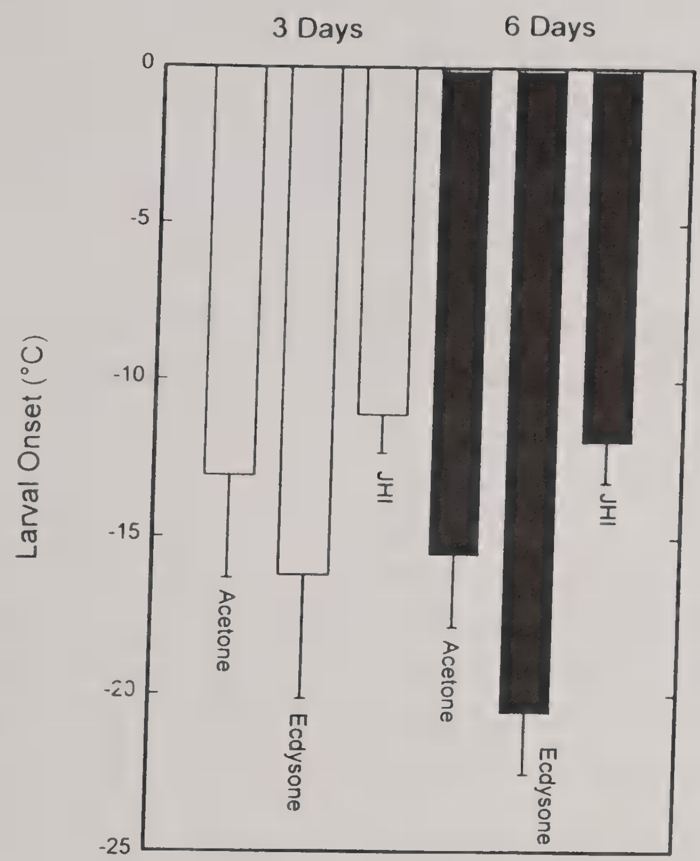


*Fig. 8.* **Effect of topical hormone application on the resistance to inoculative freezing in the mature larvae of the red sunflower seed weevil.**

## DISCUSSION

In the mature larva of the red sunflower seed weevil the acquisition of resistance to inoculative freezing appears to be associated with physical changes on its surface. These changes effectively make the larva impermeable to water and include a covering over of the spiracles by a waxy coating, reduction in respiration and an accumulation of hydrophobic lipids on its surface. This study suggests that these changes are at least in part under the control of the endocrine system. Since the larva is not freeze-tolerant this coordinated sequence of changes is crucial to its survival in a moist and cold environment. It also makes the larva potentially vulnerable to any treatment, e.g. hormone analogs, that undermine their resistance to inoculative freezing. Future studies will be geared to determining if hormone treatments affect surface lipids, respiration, spiracle structure and survival at low temperatures.

## REFERENCES

1. Rojas R. R., Charlet L. D. and Leopold R. A. (1991) Biochemistry and physiology of the overwintering in the mature larva of the red sunflower seed weevil, *Smicronyx fulvus* LeConte (Coleoptera: Curculionidae). J. Insect Physiol. 37, 489-496.

2. Rojas R. R., Charlet L. D. and Leopold R. A. (1992) A differential scanning calorimetric analysis of inoculative freezing in an insect. Cryo-Letters 13, 355-362.

# Using DNA Technology to Identify Sunflower Pests and Their Parasites

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Biological control of sunflower pests has the potential to be a major component of an overall IPM (Integrated Pest Management) strategy that reduces reliance on general insecticides. Adequate knowledge of relationships of pest species and their natural enemies, recognizing strains or populations, and tracking the movement of insects are all crucial for maximizing the success of safer control programs. The use of natural insect predators and parasites has the potential of being a self-perpetuating control regimen that would not have to be repeated every few weeks once the populations are established. Reliable identification of both adult and immature stages of insects is also needed to evaluate and use both IPM and biological control measures. Protection of biodiversity, especially of insect parasites and predators, is also very important. A full description of the biodiversity is necessary before it can be protected.

The collection of pests that attack commercial and native sunflowers in the great plains area includes the sunflower beetle, three weevils (red seed weevil, gray seed weevil, and stem weevil), the sunflower moth (*Homeosoma electellum*) the banded sunflower moth *(Cochylis hospes)* and a sister species *(Cochylis arthuri)*. Associated with these pests is a group of hymenopteran parasites, some of which may be species specific. As many as 6 parasites have been recovered from the banded moth, with two parasite species (*Glypta sp. & Chelonus sp.*) being relatively common. Both the red and gray seed weevils have parasites that have been identified (2, 3, 12).

All of the insects in the complex can be difficult to distinguish from closely related species in the larval and pupal stages, and the parasites can be difficult to identify even as adults. The parasites are tiny Hymenoptera that can present identification problems even as adults, primarily because many of them are tiny and the group is not well defined either taxonomically or biologically. The vast majority of Hymenoptera utilize a parasitic life style. Although more than 100,000 species of Hymenoptera have been described, it has been estimated that more than 75% of the parasitic species have not been identified. Parasitic Hymenoptera have the capability to respond quickly to an

outbreak of their phytophagous hosts, however under stable equilibrium conditions the population density of the parasites may be very low (7)

Currently, considerable practice and expertise is required to identify the species of parasites as adults. As immature insects, it is impossible. However, determining the rate and efficiency of parasitism is best done by dissecting larvae out of the hosts. Getting adult parasites to emerge often requires a long wait and a diapause where mortality can be high, potentially skewing the results. In addition the parasite kills the host before the host becomes an adult and some host larvae also cannot be distinguished (4). We need to be able to accurately identify both the immature parasite and the host it came from.

DNA markers are ideal for this type of research. Unlike many other measurable characters, DNA markers are not affected by the environment, diet, or developmental stage of the insect. DNA is the primary genetic material. Therefore the genotype rather than the phenotype is examined. Controlled breeding is unnecessary. In fact no prior knowledge of the genetics of a species is required for successful recovery of DNA markers.

## Types of DNA Markers

Methods of analysis with DNA include restriction fragment and restriction site comparisons (collectively called RFLP analysis), nucleotide sequencing, and size variation (e.g. micro satellites, mini satellites, random amplified polymorphic DNA or RAPDs, DNA amplification fingerprinting or AFLP)(1).

Variations of the polymerase chain reaction (PCR) are an invaluable tool for all of these DNA manipulations either as the basic component of the process itself or as a means to speed up the process and facilitate the selection of specific gene regions for analysis (5).

Our goal is to obtain DNA markers for identification, biosystematic comparisons and assessment of geographical or host population diversity in the sunflower insect complex. Initial emphasis has been on the mitochondrial DNA molecule (mtDNA), which has proven to be a very useful tool for this purpose. It is small, found in multiple copies per cell, does not undergo recombination, and is maternally inherited (i. e. effectively haploid).

We are looking for restriction site differences (RFLPs) in the mtDNA that could be diagnostic for a population or species. To facilitate this, specific regions of the mtDNA have been amplified using the polymerase chain reaction and the amplified segment has been cleaved with various restriction endonucleases (PCR-RFLP) (8, 13). The complete mitochondrial genome (16 kb = 16,000 bp) has been sequenced for several species of

insects including: honeybee, *Drosophila*, *Anopheles* mosquito, locust. As a result of this sequence information, conserved regions have been identified that are homologous in several species. These shared sequences form the basis of PCR primers that are sometimes referred to as "universal" to distinguish them from "species-specific" primers. PCR primers are used in pairs to amplify the region between them. We have used primers that amplify portions of the 12S rRNA, 16S rRNA, COI and COII genes as fragments ranging from about 400 bases to about 1700 bases.

We have also developed a procedure that makes it possible to amplify large portions of the mtDNA in one PCR reaction. This long-PCR technique can yield PCR products from 1500 bases up to 14,000 bases. With two long overlapping fragments, the complete mt-genome is available for analysis from different insect species (9).

**DNA Based Identification of Sunflower Weevils.**

Three species of weevils have become pests in commercial sunflowers. The red seed weevil (RSW) and the gray seed weevil (GSW) have similar habits. Their distribution overlaps but RSW seems to be more common in the northern Great Plains, while GSW is more common further south. The red and gray seed weevil are quite distinct as adults, but as larvae the only difference may be size which is not reliable (4). DNA markers can be used to easily resolve this problem. The sunflower stem weevil (STW) has different habits and is not so readily confused with the seed weevils. PCR-RFLP fragments clearly differentiate all three species.

Table 1 shows a summary of some of the restriction fragment data for the COI-COII region (~1500 base pairs of the mitochondrial cytochrome oxidase I and II genes) using adult weevils. The fragment sizes are given in nucleotide base pairs. RSW is the red seed weevil, GSW the gray seed weevil & STW the stem weevil. RSW and GSW share restriction patterns for *Mbo*I and *Dra*I but differ for the other two enzymes. Some intraspecific restriction site polymorphisms were also observed in these weevils despite the very restricted sample survey. These are indicated by the discovery of two AluI fragment patterns in both the RSW & STW. The existence of polymorphism in the very narrow collection suggests that these markers may be suitable for population and phylogeographic studies. An examination of additional genes and a survey of both species over a wider geographic area is needed to give a more definitive description of intraspecific variation. Since it has been demonstrated that all stages of insect development from eggs through adults have the same PCR-RFLP patterns, markers that distinguish the adults will also distinguish immatures (8).

Table 1.

PCR-RFLP AMONG SPECIES OF SUNFLOWER WEEVILS
Cytochrome Oxidase I & II Region of mtDNA

| Enzyme | Species | Fragment Size (base pairs) |
|---|---|---|
| *Mbo*I | RSW | 530, 330, 300, 270, 200 |
| | GSW | 530, 330, 300, 270, 200 |
| | STW | 500, 320, 240, 180 |
| *Dra*I | RSW | 850, 650 |
| | GSW | 850, 650 |
| | STW | 560, 520, 350 |
| *Mbo*II | RSW | 750, 350, 340, 85 |
| | GSW | 750, 740, 85 |
| | STW | 850, 700 |
| *Alu*I | RSW | 650, 360, 280, 165, 145 |
| | | 650, 500, 280, 165 |
| | GSW | 800, 600, 210 |
| | STW | 870, 490, 100 |
| | | ~1400, 100 |

A determination of whether each weevil has characteristic parasites or whether several parasites visit both weevils would require diagnostic markers for both the weevils and the parasites. We have preliminary results that confirm the existence of at least two parasites. Using Hinfl restriction enzyme and the COI-COII DNA from adults of the RSW, GSW and two parasites (*Triaspis sp. & Urosigalphus sp.)*, it appears that all four species are recognizable. Adult parasites were collected as they emerged from weevil larvae that had been held in the laboratory over the winter. Since adult parasites were used, we have no information as to whether the parasites can be found in both hosts. If only one species of weevil emerged from the same container as a particular parasite species, it is likely they were the host. However, this can only be confirmed by removing immature parasites from their hosts and testing both for diagnostic markers.

Comparing more extensive PCR-RFLP results indicates a very limited sequence diversity between RSW and GSW, about 1% (Very preliminary data using long PCR-RFLP suggests that this diversity level could be slightly higher). This level of divergence

is quite low and is more frequently found within species than between species. These two species are definitelly closely related, perhaps even sibling species. The STW is more distantly related to both of the seed weevils.

**DNA Based Identification in The Banded Moth Complex**

In the banded moth complex there are two congeneric species of moth, again indistinguishable as larvae, and at least six parasitoids. Two of the parasitoids are fairly common and one other is actually a hyper parasite, a parasite of one of the other parasites (12). Little is known about how the moth parasites are related to each other or whether some are more closely related to the weevil parasites. Do parasites evolve in concert with particular hosts or do they evolve in concert with the environment where their hosts are found (e.g. sunflowers)? Comparison of DNAs can find correlations that can begin to answer these questions.

Restriction fragment patterns from the COI-COII region using Dra I for the two moths, *Cochylis hospes & C. arthuri* along with two known presumptive *C. hospes* parasites, *Glypta sp. & Chelonus sp.* are shown on Table 2. All four appear distinctive.

Table 2

PCR-RFLP IN BANDED SUNFLOWER MOTH & PARASITES
Mitochondrial COI-COII Region

| Enzyme | Species | Fragment Size (base Pairs) |
|---|---|---|
| *Dra*I | *Cochylis hospes* | 600, 500, 260, 120 |
| | *Cochylis arthuri* | 650, 600, 120 |
| | *Glypta sp.* | 950, 395, 220 |
| | *Chelonus sp.* | 1200, 260, 135 |

Another approach to DNA genetic markers that we plan to investigate is random amplified polymorphic DNAs (RAPDs). RAPDs employ single short primers for the PCR reaction producing a pattern of fragments ranging from about 200-3000 base pairs. RAPDs sample the nuclear genome and each different primer sequence yields a different pattern of DNA fragments. Previous work with RAPDs in parasitic hymenoptera and beetles has demonstrated that they can be useful not only for discriminating species, but also strains and geographical variants (10, 11). RAPDs provide another tool for

recovering useful DNA variability.

Well defined DNA markers can have a significant impact on the search for new parasites. The results can also be used to help determine which parasites are most efficient. With mtDNA PCR-RFLP the same genes can be examined in all species, population variability can be assessed, historical movements can be traced through maternal lineages, biosystematic relationships can be inferred, new species of parasites (including sibling species and "cryptic" species) can be identified. All of this can enhance the possibilities for and effectiveness of biological control.

**Very Important Cooperators**

This work was made possible by the indispensable efforts of **Dr. Larry Charlet** and **Ms. Theresa Gross** who diligently collected, held through diapause, and provided us with emerged adults from the seed weevil and banded moth insect complexes. We are also indebted to **Dr. John Barker** and **Ms. Sharon Grugel** for maintaining the laboratory colony of stem weevils and sharing some with us. Finally **Mr. Craig Mueller** deserves commendation for his skill with the PCR-RFLP process.

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# Relative Efficacy of Different *Bacillus thuringiensis* Formulations against Larvae of the Banded Sunflower Moth (Lepidoptera:Tortricidae)

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## Introduction

The banded sunflower moth (BSM), *Cochylis hospes* (Walsingham), has been one of the major pests of cultivated sunflower, *Helianthus annuus* L., in the Northern Great Plains (Westdal 1949, Schul z 1978, Charlet and Busacca 1986). Its larvae feed and develop on pollen and florets, and in later instars achenes. Although various synthetic organic insecticides are the principle control practices employed, these chemicals have created problems because of their effects on non-target organisms, such as natural enemies and pollinators. As an alternative to chemical pesticides, interest in the use of microbial pesticides, such as *Bacillus thuringiensis* Berliner, for insect pest management has increased. *B. thuringiensis* (Bt) offers a degree of specificity and safety not found in other chemical pest control methods. Although various formulations of Bt have been used against a number of lepidopterous species including the sunflower moth, *Homoeosoma electellum* (Hulst), there is no information available on efficacy of Bt against larvae of the banded sunflower moth. Because different formulations of Bt are based on different strains and subspecies, their activity against BSM may differ.

The objective of this study was to evaluate relative efficacy of various formulations with different strains and subspecies of Bt against larvae of the banded sunflower moth.

## Materials and Methods

**Field Location**. The experiment was conducted in 1995 at the North Dakota State University's Experimental Station, Prosper, North Dakota. Plots were seeded on May 24 with sunflower cv: H-894 in soil that had been treated before planting with an application of trifluralin for weed control. Plots were four rows wide by 10 m long, with 76 cm between rows and 30 cm between plants. This plot size was an equivalent to 47,000 plants per hectare. Each plot was buffered by a 2.5 m fallow area between treatments and 3.5 m between blocks.

**Sources of Chemicals**: Treatments were six commercially available Bt products, and two other chemicals plus an untreated control. The insecticides and their sources were: 1) Dipel® 2X WP, and 2) XenTari® TM (Chemical and Agricultural Products Division, Abbot Laboratories, North Chicago, IL); 3) Javelin® WG (Sandoz Crop Protection Corporation, Sandoz Ltd, San Diego, CA); 4) Thuricide® AF (Southern Mill Creek Products Co., Tampa, FL); and 5) Cutlass® WP, and 6) Condor® OF (Ecogen, 2005 Cabot Boulevard West, Langhorne, PA), 7) Bee Here (a mixture of Troy-Bt, EPA Reg. No. 53871/6, and *Beauveria bassiana*, EPA experimental use permit No. 53871-EUP-1 (Troy Biosciences, Inc., Phoenix, AZ 85009), and Bee Fermone (Fermone Corporation, Inc., Phoenix, AZ 85009), 8) Asana® (E. I. Du Pont De Nemours & Company, Agric. Products Walkers Mill, Barley Mill Plaza, Wilmington, DE 19880), an standard insecticide, and 9) an untreated control. All treatments were applied with a tractor - mounted boom sprayer to plots at R 5.1 growth stage. An equivalent of 117 liters of water per hectare was used in the application of the treatments. Insecticides application rates are presented in Table 1.

**Data Collection and Analysis:** : At growth stage R9 four randomly selected sunflower

heads from the middle two rows of each plot were hand-harvested. The heads were placed in a drying oven at $66^0$ C for approximately one week. After drying, each head was hand-threshed, cleaned, and evaluated separately. Consecutive, randomly selected 100 seed samples were used to estimate the percent damaged seed, seed set, and 100 seed weight.. Then the seeds from the four sunflower heads per plot were pooled and used to determine seed yield (g/head). Data were subjected to analysis of variance using the PROC ANOVA (SAS Institute 1995) and means were separated with Duncan's multiple range test ($P < 0.05$).

## Results and Discussion

There were significant differences in head diameter between all treatments (Table 1). An increase in diameter could significantly increase seed yield between treatments. Sunflower treated with the Bt products and Asana significantly reduced the percent seed damage compared to the untreated control. The Javelin and Condor treatments had the lowest percent seed damage of Bt products and gave control equivalent to Asana. Sunflower heads treated with Dipel showed significantly lower percent seed set compared to the other treatments. However, there were no significant differences in seed set between the other treatments. Sunflower heads treated with Cutlass WP had a significantly higher seed weight than those treated with Condor and Asana, but not from the other treatments. Javelin and Thuricide treated sunflower heads had a significantly higher yield than those treated with Condor, but they did not differ from the other Bt treatments and Asana. Javelin treated sunflower heads had both low damage, and high yield. Thus, Javelin gave the best combination of results and is a desirable biological insecticide to use against BSM larvae because its safety towrards natural enemies and pollinators, bees.

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**Table 1: Comparisons six Bt formulations of strains and other chemicals against larvae of the banded sunflower moth on damaged seed, seed set and weight, and yield, Prosper, Fargo, ND 1995**

| Treatment | Dosage [g (ml)/ L) | Diameter (sq. cm) | Damaged seed (%) | Seed set (%) | 100 seed weight (g) | Yield (g/head) |
|---|---|---|---|---|---|---|
| Dipel 2X | 1. 5 | 48.1 bc | 9.1 bc | 93.5 b | 3.6abc | 29. bc |
| Javelin WP | 1. 5 | 54.6 ab | 7.2 c | 96.9 a | 3.7 abc | 42.2 a |
| Xentari M | 2. 3 | 51.0 abc | 8.6 bc | 95.6 a | 3.6 abc | 38.3 abc |
| Thuricide AF | 12.1 | 55.6 ab | 7.6 c | 95.7 a | 3.7 abc | 44.6 a |
| Cutlass WP | 1.9 | 60.8 a | 10.3 b | 96.0 a | 4.1 a | 41.8 ab |
| Condor OF | 1.9 | 44.3 c | 7.3 c | 95.5 a | 3.3 c | 28.4 c |
| Bee here [a] | 2.3 | 53.2 abc | 8.4 bc | 95.9 a | 3.7 abc | 37. abc |
| Asana | 2.1 | 51.3 abc | 7.1 c | 95.8 a | 3.5 bc | 35.4 abc |
| Control | - | 56.1 ab | 14.3 a | 96.4 a | 3.9 ab | 41.3 ab |

[a]) a mixture of Troy-Bt, *Beauveria bassiana*, and Bee Fermone

# MICROSAMPLING CAPITATE GLANDS OF SUNFLOWER, AND ITS APPLICATIONS FOR DETERMINING RESISTANCE TO THE BANDED SUNFLOWER MOTH, (*Cochylis hospes* Wlshm).

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The localization of glandular trichomes in the fused anther appendages of numerous oilseed producing sunflower varieties makes it possible to use a microsampling technique to investigate for the presence of secondary lipophilic metabolites, called sesquiterpene lactones (SQL), contained in the glands that may be involved in host plant resistance to the Banded Sunflower Moth (*Cochylis hospes* Wlshm.) and other insect pests.

## Materials and Methods

Florets of four different plant introductions (PI 480471, PI 3291, PI 172906, (with three different seed colors, black, white, and striped)), and hybrid '894' (used as susceptible check) were manually collected from the heads using a small forceps. From each floret 60 glandular trichomes were mechanically removed from the apex of the fused anther appendages (Figure 1) with a minuten probe. In accordance with Spring (1989), immediately after being removed from the anther appendages the glands were dissolved in 50 ul of 100% MeOH. If the glands couldn't be tested immediately after incorporation into the MeOH, this solution was refrigerated until needed. When testing the glands, 50 ul of distilled H2O was added to each sample and then the sample was centrifuged at 10,000 rpms for 3 minutes. The samples were then directly injected into an isocratic HPLC system (MeOH-H2O, 1:1 v/v, 1 ml/min.). A Shandon

Hypersil® ODS reverse phase column (4 x 250 mm, 5 um) was used, with peak detection performed at 225 nm. Dimethylphenol (DMP) was used as an internal standard, with a total elution program time of 29 minutes. The chromatographs resulting from the HPLC test were then compared to a standardized mean chromatograph (Figure 2) (Spring, 1990) that exhibits the average elution times for SQLs found in several *Helianthus* species.

**Results and Discussion**

Figures 3 through 9 are approximate reproductions of the chromatographs from the HPLC samples forced through the system. The chromatographs of the tested plant introductions are overlaid on Spring's typical elution diagram (Figure 2) to show the comparison between the chromatographs. The two large peaks before 5 minutes are chemicals that are common in sunflowers, but have not been shown to be involved in resistance. Spring's elution diagram is shown in figures 3 through 9 as a thin black line. While similarities between peak elution times are indicative of the same chemical, it cannot be stated that these are the same chemicals, due to the fact that the chemicals were never chemosystematically identified. Therefore, by inference it may be said that there is a good likelihood that similar elution times are the same SQLs.

Figure 3 shows the elution diagram for hybrid '894'. There are similarities in the peaks eluting at approximately 7,8, and 9 minutes. There is also the addition of a peak at approximately 12.5 minutes. Figure 4 shows the elution diagram for PI 3291. This shows similarities between peaks 2, 3, and 4. There seems to be a time shift with peaks 1 and 6. This may be caused by differences in the equipment used for the standard diagram and the tested plant introductions. Figure 5 shows the elution diagram for PI 480471. This exhibits excellent similarities in all the peaks. Figure 6 shows the elution diagram for PI 172906 (white seed). Here there are some similarities with a significant time shift for elution that may also be due to equipment differences. Figure 7 shows the elution diagram for PI 172906 (black seed). There is a large reduction in the peak similarities with the loss of 1, 4, and 6. In figure 8 (PI 172906 striped seed) there seems to be a

loss of peak 3 and 5 if the elution time is adjusted to the left to compensate for equipment differences. In figure 9, the three different seed colors for PI 172906 are compared to each other (and Spring's standard). This diagram exhibits the great heterogeneity found in plant introductions, and how resistane can vary within a single plant introduction. Previous experiments have linked morphological traits with expression of resistance to other crop pests (Wilson, et.al 1993), this may be an example. According to the number of SQL peaks attained from the HPLC samples, white seed showed to have more of a chance of having the SQLs than the black or striped seeds, therefore it may be more resistant.

With morphological expression as an indicator of host plant resistance in mind, there is noticeable differences in glandular trichome morphology. The contents of each gland (i.e. SQLs) are protected from UV light by pigments. These pigments have been identified in the case of clear glands as flavonoids. It is speculated that the purple or black glands contain anthocyanidins, and the yellowish glands contain carotenoids. Usually 2 of the three different colored glands can be found on the apex of fused anthers of each of the plant introductions tested. Table 1 shows the comparison between peaks and the colors of glands found on the anthers.

**Table 1.** Probable Pigmentation and HPLC Peaks Similar to Spring's Standard of Floral Glandular Trichomes from Different Sunflower Germplasm

| | Pigments Present | | | |
|---|---|---|---|---|
| PI # | Flavonoid | Anthocyanidin | Carotenoid | # of Similar Peaks |
| 894 | X | | X | 3 |
| PI 172906 | | | | |
| white seed | X | | X | 5 |
| black seed | X | X | | 2 |
| striped seed | X | X | | 4 |

| | | | | |
|---|---|---|---|---|
| PI 480471 | X | X | X | 6 |
| PI 3291 | X | | | 3 |

Field trials involving these lines were conducted by artificially infesting Banded Sunflower Moth eggs on R-5.5 stage sunflower heads. The ability to resist damage was assessed by counting the number of damaged seeds on each head. PI 480471 and 172906 showed minimal damage compared to the other varieties. The white seeds of 172906 showed approximately the same damage as the black and striped seeds (Ellefson, field data 1995). Table 1 shows the glands from each of the germplasms tested. This suggests that gland morphology may effect the level of resistance, but not enough is known to use these traits to screen for resistance.

Future applications of glandular trichome microsampling include: the identification of new allelochemicals that may be useful in host plant resistance research; selecting lines for feeding assays; comparing the amounts and types of allelochemicals found in different species of *Helianthus;* eventually it may be a simple and economical method to test for resistance to the Banded Sunflower Moth and other sunflower pests.

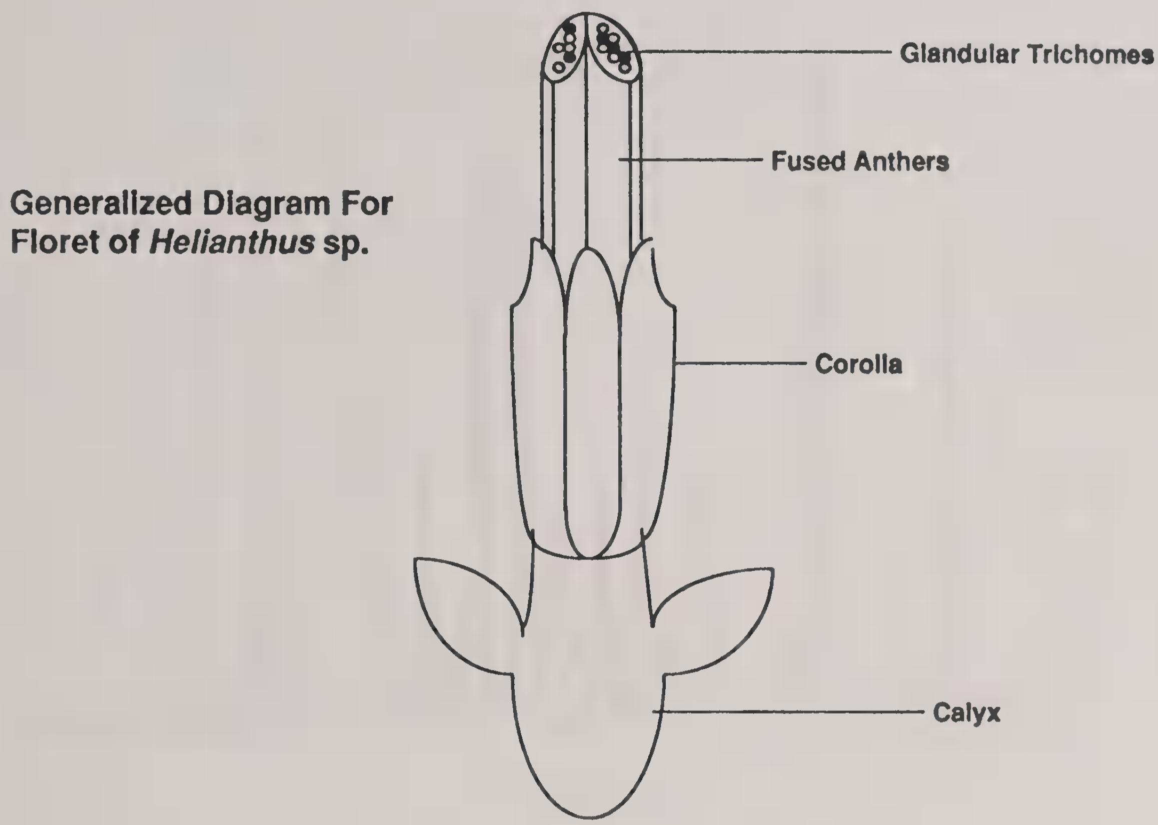


Figure 1

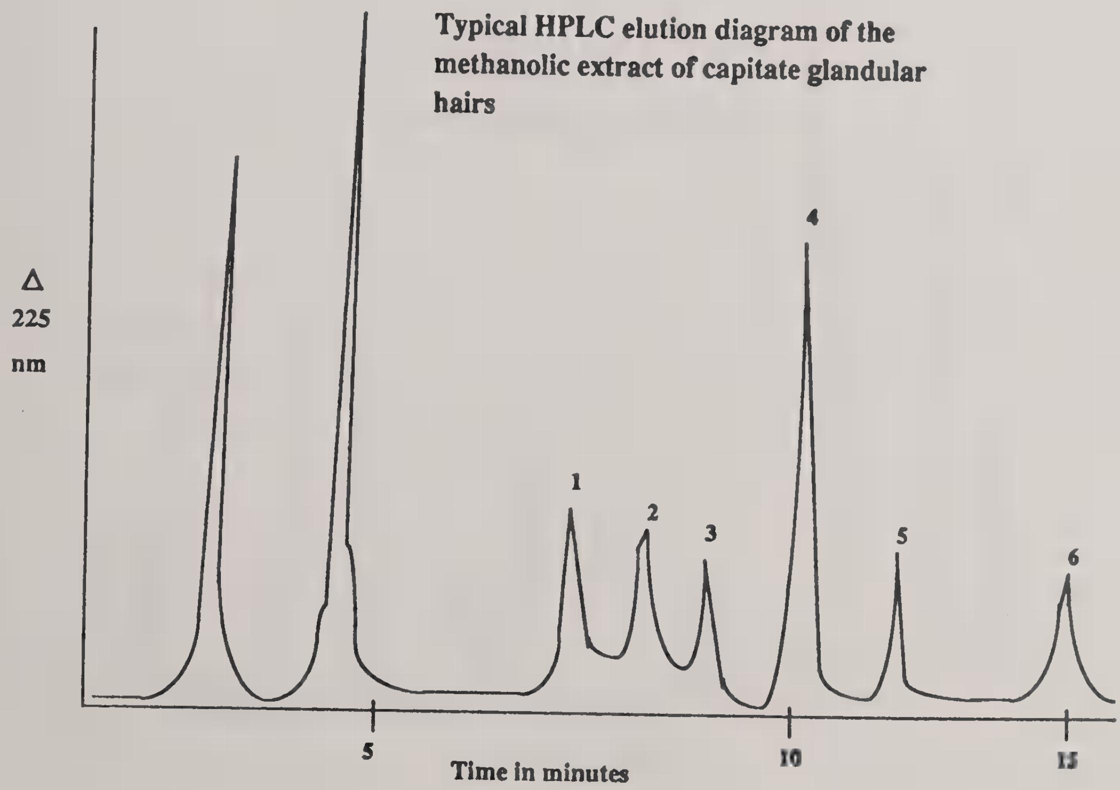


Figure 2

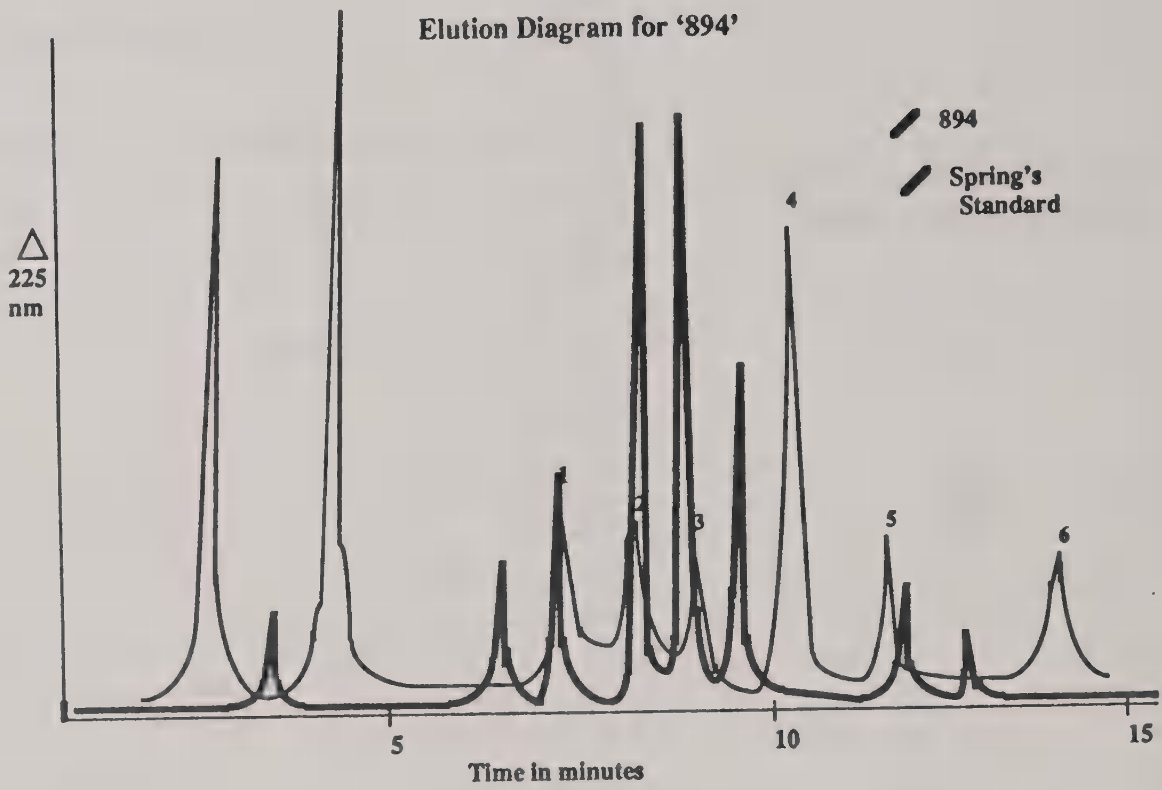


Figure 3

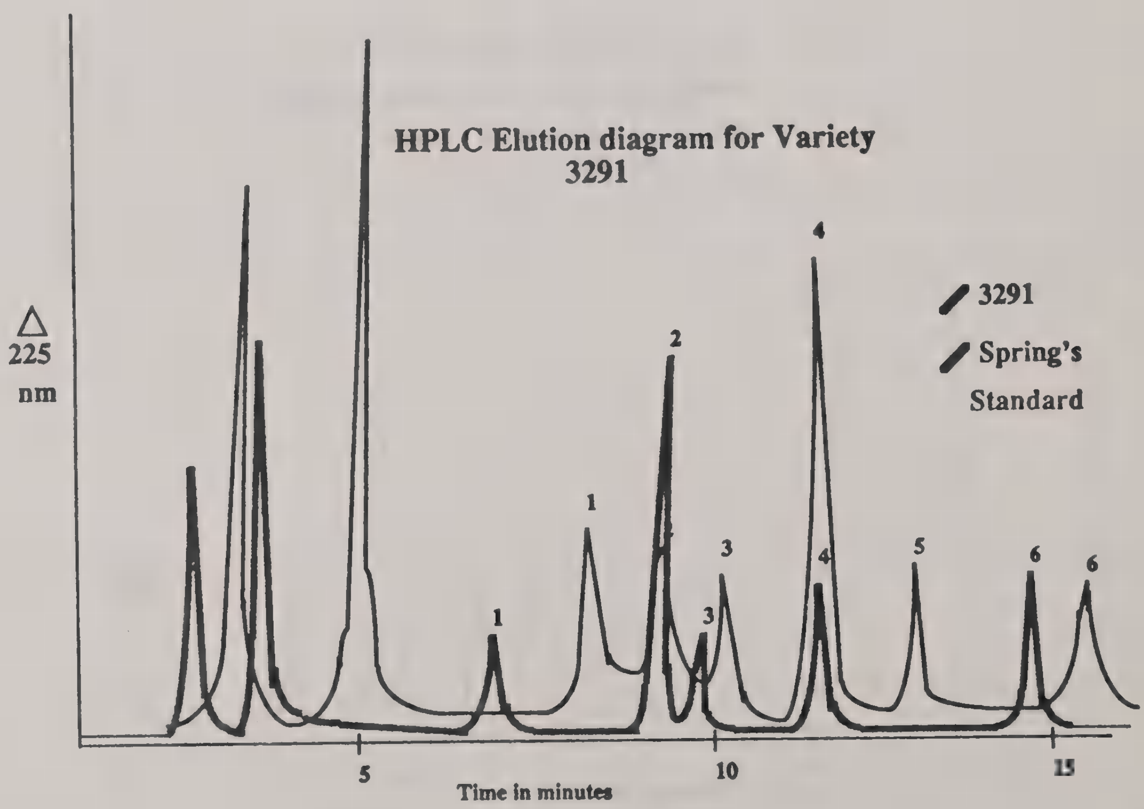


Figure 4

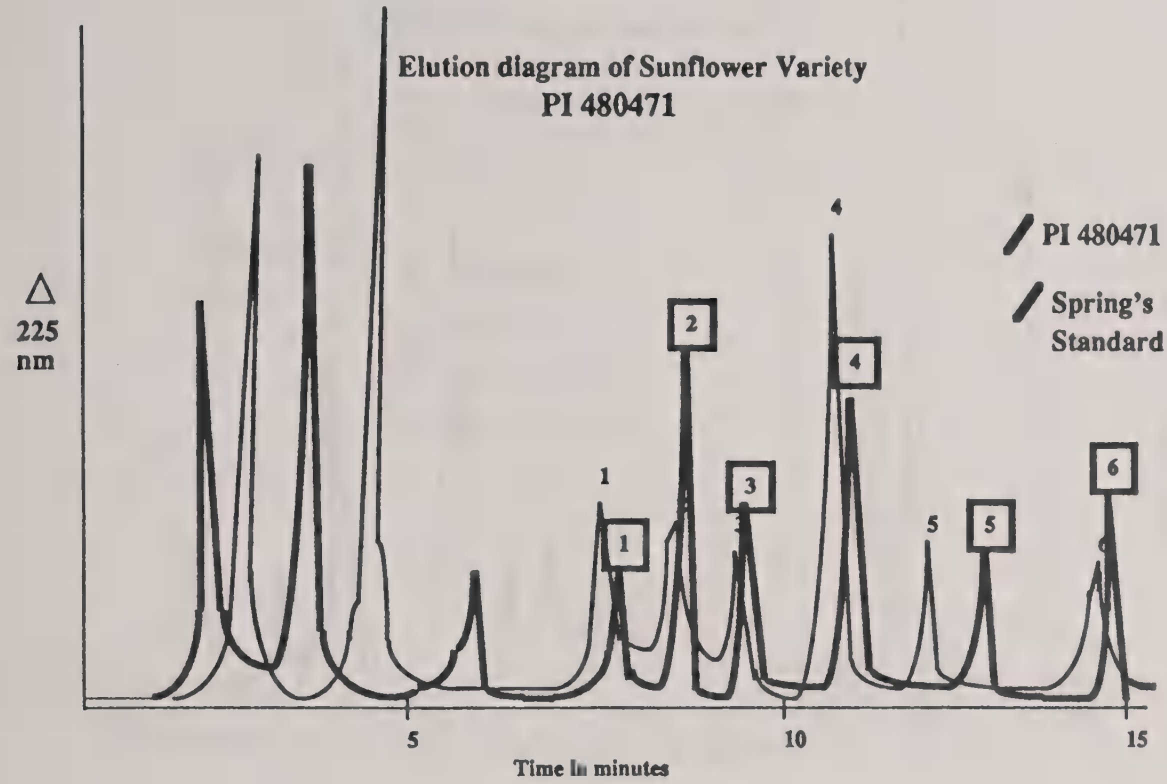


Figure 5

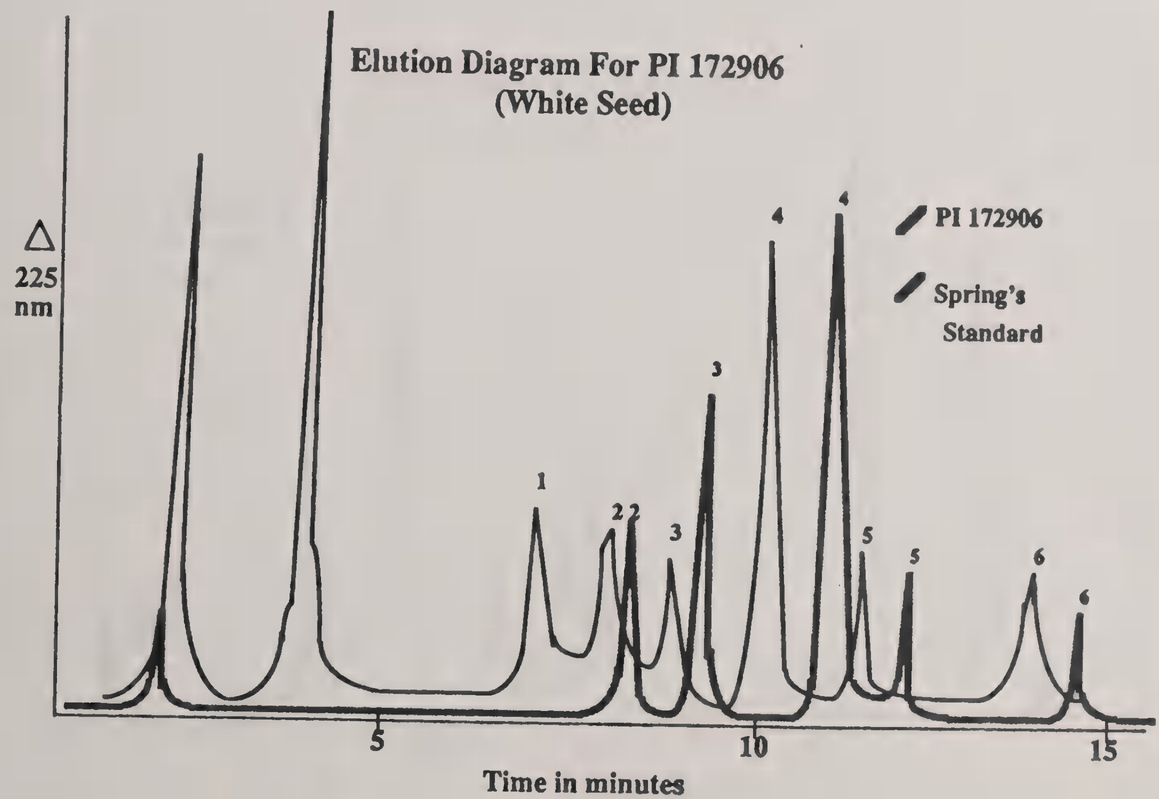


Figure 6

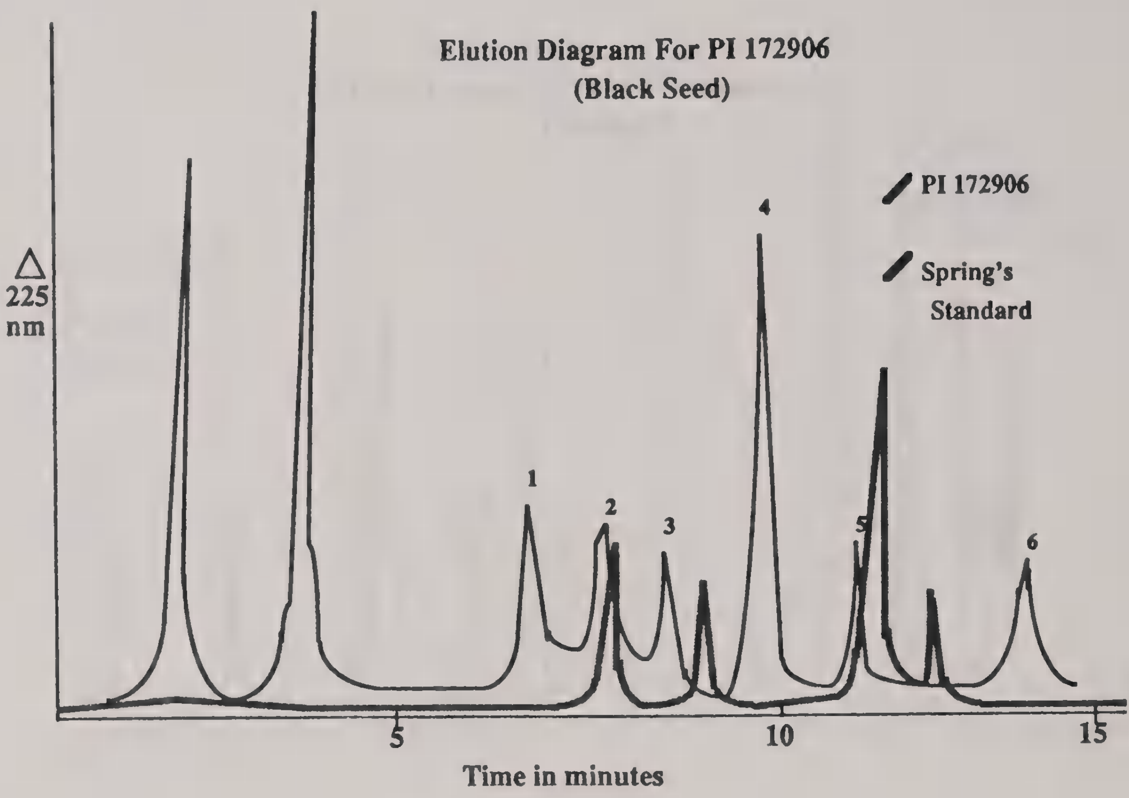


Figure 7

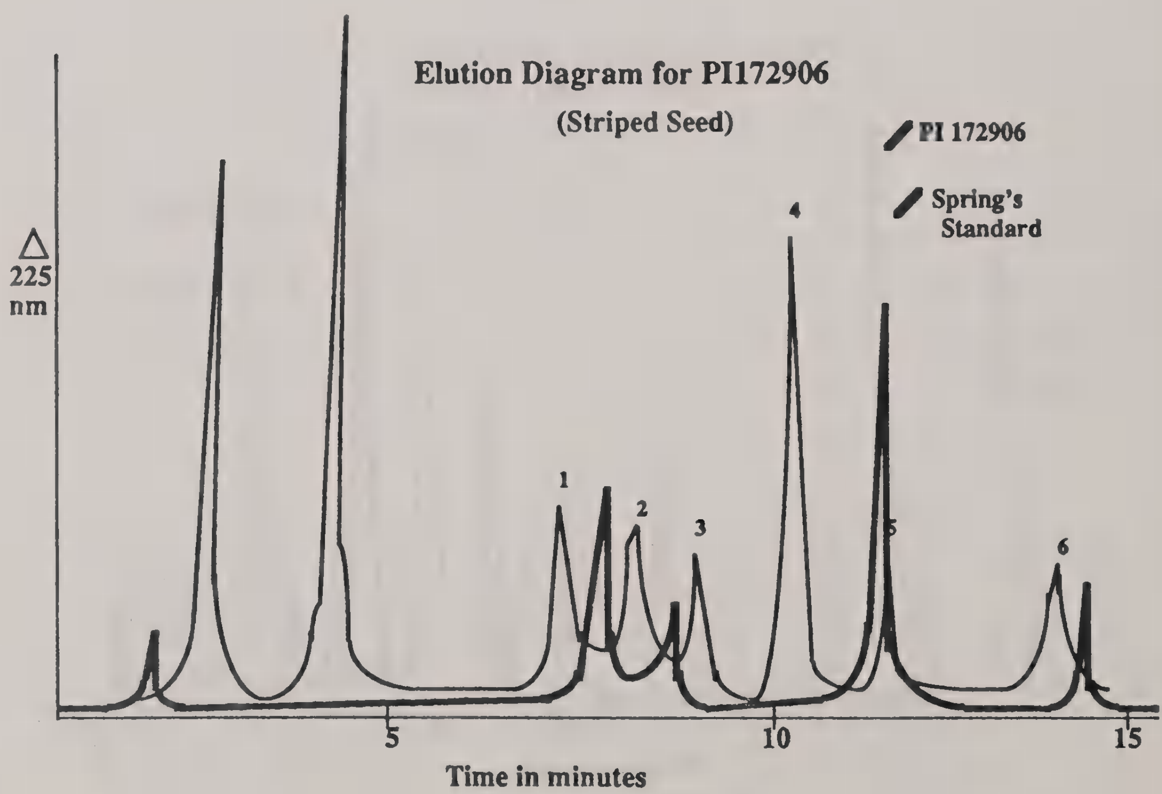


Figure 8

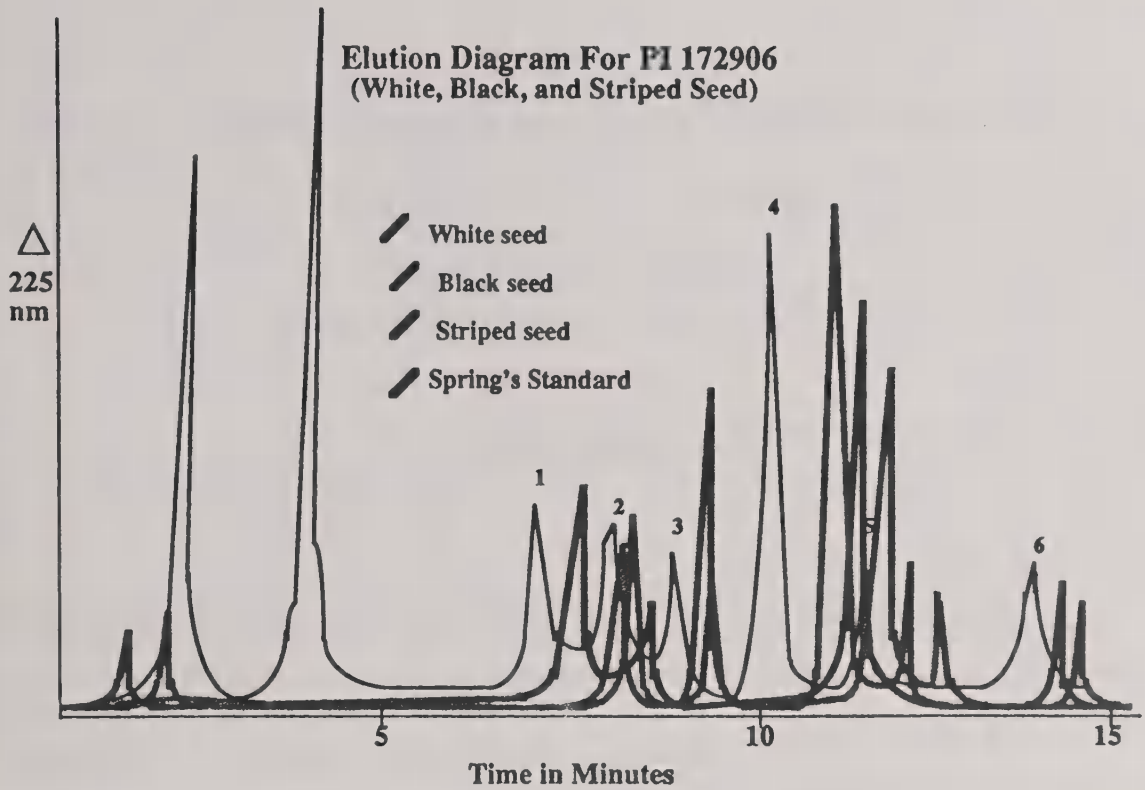
Elution Diagram For PI 172906
(White, Black, and Striped Seed)
White seed
Black seed
Striped seed
Spring's Standard
△
225
nm
1
2
3
4
5
6
5
10
15
Time in Minutes

Figure 9

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Spring, O. Sesquiterpene Lactones From *Helianthus tuberosus.* (1990) Phytochemistry. Vol. 30, No. 2 pp. 519-522.

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# Effect of Planting Strategies on Feeding by the Sunflower Beetle

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The need for resistant varieties of sunflower that can protect commercial crops against the sunflower beetle seems greater than during previous years, because the numbers of this insect have been consistently high since 1993 (Roseland, 1996). A search for resistant lines has not resulted in germplasm that would indicate major progress, but a few lines that are modestly resistant when compared to others have been found (Satter, 1996). If it were possible to use these modestly resistant lines (especially existing commercial varieties) in some strategy to enhance the effectiveness of their comparative deterrence, then perhaps such a strategy might be used agronomically in the immediate future.

Grosz and Roseland (1994) investigated mixtures of the comparatively susceptible (ST 316) and resistant (RHA 274) lines in proportions of 3:1, 2:1 and 1:1 in cage assays. Feeding on the resistant lines was decreased when resistant plants were present in cages at a ratio of 1:3 with respect to the susceptibles. However, most mixtures resulted in increased feeding over all the plants in the cage. In the present experiments, we will show that using the least preferred lines as a border around the preferred ones does offer some protection from feeding to all plants in a cage.

Four types of plantings were set up. In the first type, two lines, ST 316 and RHA 274, were random interplanted in a 7 X 7 matrix in each 6' x 6' x 8' cage. In a second type, side by side blocks of each genotype were planted. In the last two types of plantings, either susceptibles were planted in a single row around the periphery of a block of resistant plants or the converse of this arrangement, resistant ones were planted as the periphery row surrounding a block of susceptible plants. One hundred beetles were released at the four corners of each cage. Counts of feeding initiation sites were made on successive days following growth of the new leaves. Measurements of three leaf pairs were obtained. Three replicates of each experiment were accomplished.

Figure 1 shows that the amount of feeding on the susceptible genotype was greatly increased by the planting arrangement given it with respect to the resistant genotype. When the susceptible was outermost of the plant rows, feeding on the susceptibles was greater than that seen on the susceptibles of any other arrangement. When the resistant line was outermost, feeding on the enclosed susceptibles was less than in any other arrangement. There was a two-fold decline in feeding on the susceptibles when they were enclosed by resistant plants than when they formed the outer rows of the planting. Feeding on the susceptibles in the random mixtures and the side by side blocks was of intermediate value.

Figure 2 shows that total feeding on both S and R plants could be greatly altered by the arrangement of plants in the cage. Again, when the row of resistant plants was placed on the

outer margins of the block, total feeding was reduced to 65% of that when a susceptible population was on the outer margins of a resistant block. When susceptibles were planted on the outside of the block, the total feeding was increased by 20% over that in the cages with side by side resistant and susceptible blocks. We also analyzed the ratio of susceptible feeding to resistant plant feeding (also Figure 2). When susceptibles were planted on the outer margins, the insects were more than twice as likely to feed on these plants than the resistant plants in the interior of the cage planting. When resistant plants were planted on the outer margins, the insects were less likely to feed on one of the susceptibles than on a resistant line.

**Conclusions**: A significant protective effect can accrue to a block of caged plants if resistant lines are planted on the margins of the block. Feeding is reduced on both the resistant plants, which must be encountered first, and also on the susceptibles which are on the interior of such cages. In fact, a 35% overall reduction in feeding is seen between the susceptible outermost and the resistant plants outermost in terms of feeding site initiations.

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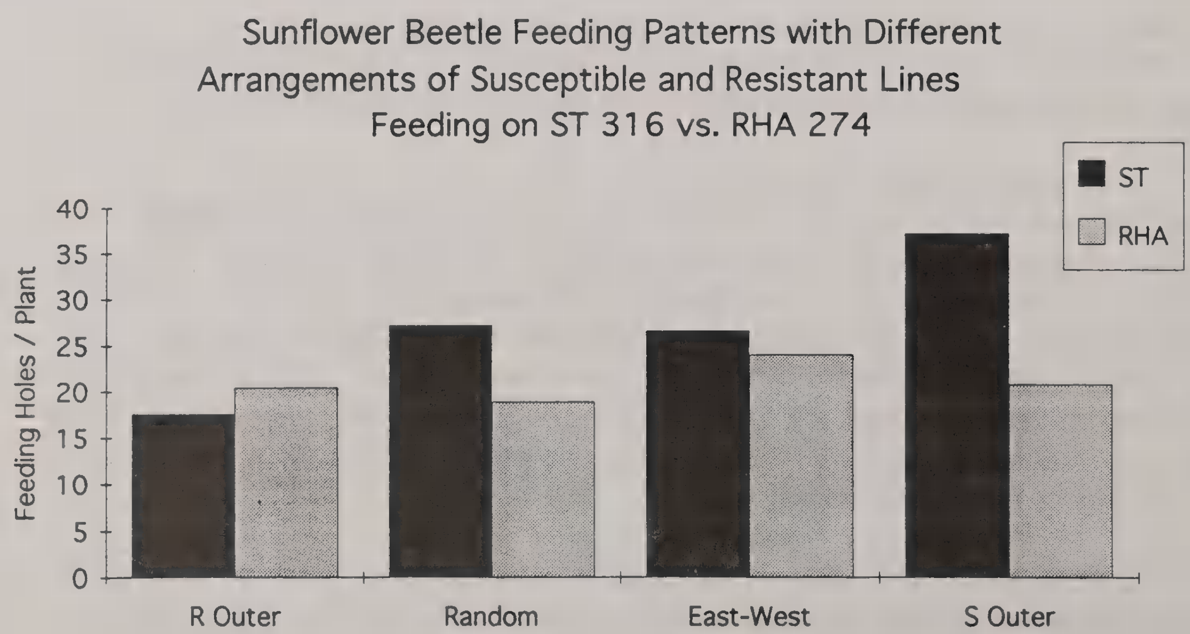


Figure 1

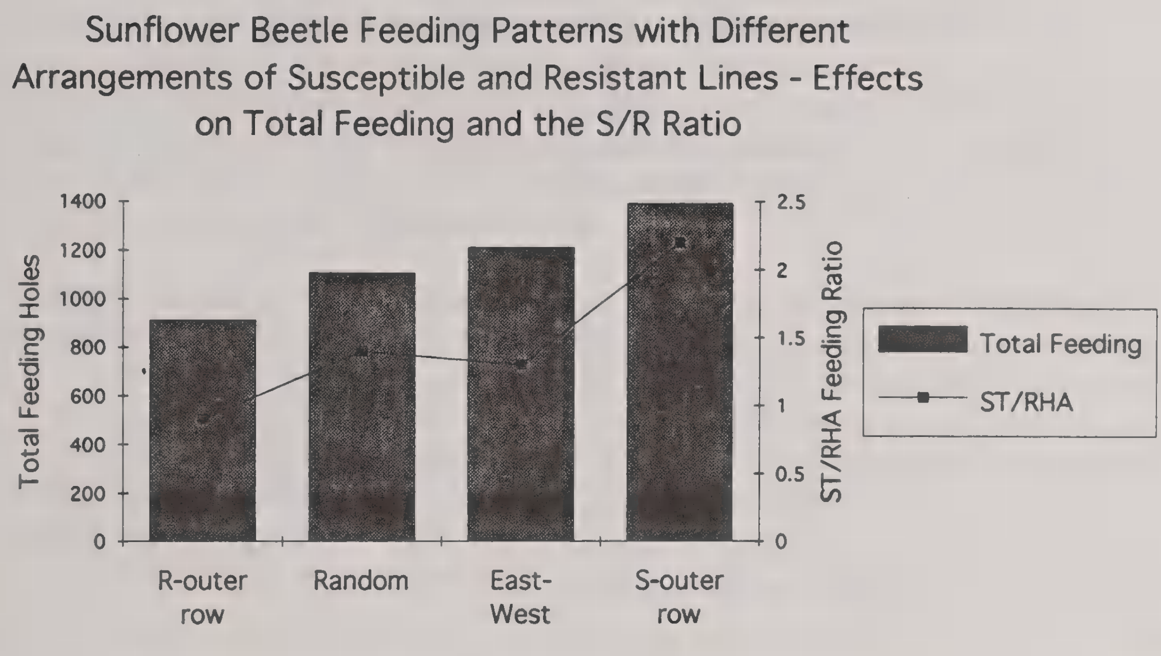
Sunflower Beetle Feeding Patterns with Different Arrangements of Susceptible and Resistant Lines - Effects on Total Feeding and the S/R Ratio
Total Feeding Holes
ST/RHA Feeding Ratio
1400
1200
1000
800
600
400
200
0
2.5
2
1.5
1
0.5
0
R-outer row
Random
East-West
S-outer row
Total Feeding
ST/RHA

Figure 2

# CONFIRM Insecticide: Chemistry and Activity

Dr. W. Eugene Thilsted
Central Region - Research and Development Manager
Rohm and Haas Company
Houston, TX

**CONFIRM®** insecticide is the first insecticide to imitate the natural insect molting Hormone, 20-hydroxyecdysone. CONFIRM insecticide works by strongly binding to the ecdysone receptor protein which, when activated, initiates the molting process. Once CONFIRM has bound to the ecdysone receptor, the caterpillar ceases feeding and produces a new but malformed cuticle beneath the old cuticle. The caterpillar, unable to shed its old cuticle, dies of dehydration and starvation. Because of it novel mode of action, CONFIRM is not expected to be cross-resistant to other insecticides, which also makes it an important tool in resistance management.

CONFIRM insecticide is a new insecticide for control of lepidopteran pests, specifically. It has no impact on the natural population of beneficial, predatory and parasitic insects for the control of other insect pests. Thus, CONFIRM insecticide fits well into integrated pest management programs offering a highly effective but "softer" selective insecticide. For example, in apple orchards treated with CONFIRM insecticide, a variety of predators and parasites including lacewings, Typhlodromus, Stethorus and Ascogaster, will be unaffected by CONFIRM applications. These predators and parasites contribute to the control of other important insects and mites and thus reduce the need for the application of insecticides or miticides for their control.

Rohm and Haas Company has evaluated CONFIRM insecticide and has determined that it poses minimal risk to human health and to the environment. CONFIRM insecticide is practically non-toxic following acute exposure. The toxicity of CONFIRM insecticide can be characterized as:

- Non-mutagenic
- Non-oncogenic
- Non-teratogenic
- Non-sensitizer
- Non-embryofetotoxic
- Non-neurotoxic

The absence of adverse findings in the completed toxicological profile of CONFIRM insecticide suggests that this insecticide does not pose an unreasonable risk to the users of handlers of the product.

CONFIRM is moderately toxic to fish, but only above its limit of solubility, and practically not-toxic to avian species through acute exposure. Most non-target terrestrial organisms are unaffected when exposed to CONFIRM insecticide in laboratory studies. In the environment, CONFIRM degrades in soil and water through microbial activity and photolysis, eventually forming carbon dioxide. In field dissipation studies, CONFIRM insecticide has shown relatively short half-life with minimal downward movement.

| **TRADE NAMES** | ***CONFIRM*** Insecticide<br>***MIMIC*** Insecticide | |
|---|---|---|
| Chemical Class | Diacylhydrazine | |
| Code Numbers | RH-5992 | RH-75992 |
| ISO Name | Tebufenozide | |
| IUPAC Name | N-tert-butyl-N'-(4-ethylbenzoyl)-<br>3,5-dimethylbenzohydrazide | |
| Chemical Abstract's Name | 3,5-Dimethyl benzoic acid<br>1-(1,1-dimethylethyl)-2-<br>(4-ethylbenzoyl) hydrazide | |
| CAS Registry Number | 11240-23-8 | |
| Melting Point (°C) | 190 to 191 | |
| Log P by HPLC | 4.25 | |
| Water Solubility | 0.83 ppm | |
| Vapor Pressure (25 °C) | 2 X 10-8 Torr | |
| Molecular Weight | 352 gram/mole | |
| Molecular Formula | $C_{22}H_{28}N_2O_2$ | |

The activity of CONFIRM insecticide is generally expressed following ingestion by the target larvae. Consequently, the timing of application is dependent on the feeding behavior of the target pest. For foliar-feeding larvae, application made while active feeding is occurring will be effective. For cryptic-feeding larvae, application must be made prior to the time that surface feeding occurs. CONFIRM insecticide has good residual activity and should protect the crop following the peak flight of each generation. In the case of prolonged adult flight, follow-up applications may be required. For season-long insect pressure, applications should be made at a frequency that protects new growth.

CONFIRM insecticide formulations currently in development include clay-based wettable powder in water-soluble bags, low AI dry granules, liquid flowables, and specialized ultralow-volume aerial formulations. The use of spray adjuvants that insure coverage and adhesion of CONFIRM insecticide to the plant surface is recommended.

**Mammalian Toxicology Studies with CONFIRM Insecticide**
**Acute Profile**

| | | |
|---|---|---|
| **Technical** | | |
| Acute oral (rat) $LD_{50}$ | >5000 mg/kg | Practically non-toxic |
| Acute dermal (rat) $LD_{50}$ | >5000 mg/kg | Practically non-toxic |
| Acute inhalation (rat) $LD_{50}$ | >4.3 mg/L | |
| | | |
| Primary dermal irritation | | Non-irritating |
| Primary eye irritation | | Non-irritating |
| Dermal sensitization | | Non-sensitizer |
| | | |
| **2F formulation** | | |
| Acute oral (rat) $LD_{50}$ | >5000 mg/kg | Practically non-toxic |
| Acute dermal (rat) $LD_{50}$ | >5000 mg/kg | Practically non-toxic |
| Acute inhalation (rat) $LD_{50}$ | >2.7 mg/L | |
| | | |
| Primary dermal irritation | | Non-irritating |
| Primary eye irritation | | Slightly irritating |
| Dermal sensitization | | Non-sensitizer |

# The Biology and Management of the Sunflower Stem Weevil, *Cylindrocopturus adspersus*: Past, Present, and Future.

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## Introduction

The sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte) (Coleoptera: Curculionidae), is an insect pest that has caused economic damage to sunflower in Texas, North Dakota, South Dakota and Minnesota (Schulz 1978, Rogers and Jones 1979, Charlet et al. 1987). The sunflower stem weevil has been reported from most states west of the Mississippi River and into Canada. It was first described in 1876 from specimens collected in Texas and California (LeConte 1876). The weevil was first noted as a pest of sunflower by Newton (1921) from severely wilted plants in fields grown for silage in Colorado. In the southern Great Plains, Phillips et al. (1973) noted that larval densities were about 40 per stalk with one plant harboring 177 larvae. Schulz (1978) reported that the first sunflower stem weevil infestation occurred in North Dakota in 1973. The field had sustained an 80% yield loss due to plant lodging. The weevil has also been found infesting sunflower stalks in Saskatchewan (Arthur and Mason, 1990). Recently (1993-1994) damage has been reported from the central plains region including eastern Colorado, western Kansas, and Nebraska (Armstrong 1996).

Adult sunflower stem weevils are 4 to 5 mm long and grayish-brown with varying-shaped white spots on the elytron (wing covers) and thorax. The snout, eyes, and antennae are black. The larvae are 5 to 6 mm long at maturity. They are creamy-white with a small, brown head capsule and are legless. They are normally in a curled or C-shaped position within the sunflower stalk (Casals-Bustos 1976, McBride et al. 1990, 1994). The number of larval instars appears to be variable and 5-7 have been noted (Barker and Charlet 1992).

In addition to cultivated sunflower, the sunflower stem weevil has also been collected from different species of native sunflowers, including *Helianthus annuus*, *H. pauciflorus*, *H. petiolaris*, *H. tuberosus*, and *H. maximiliani* (Charlet 1983b, Charlet et al. 1992). Records also include ragweed, pigweed, Russian knapweed, lambsquarter, golden ragwort, perennial sowthistle, red clover, cocklebur, kochia, and sugarbeets (Mitchell and Pierce 1911, Pierce 1916, Goeden and Ricker 1975, 1976, Casals-Bustos, 1976, Schulz 1978).

Surveys of cultivated sunflower fields in North Dakota, South Dakota and Minnesota showed an average density of 2 larvae per stalk at 18 locations in 1979 and 1.6 larvae per stalk at 47 locations in 1980. About 50% of the stalks examined were infested with sunflower stem weevil larvae (Charlet 1983a). Arthur and Mason (1990) sampled sunflower fields in Saskatchewan and recorded an average of 6.0 larvae per stalk from 10 locations in 1984, 0.9 larvae per stalk from 13 locations in 1985, and 0.2 larvae per stalk from 9 locations in 1986. Samples from research plots in southeastern North Dakota from 1980 to 1994 (no samples

were made 1986-1988) showed larval densities from a high of 107.9 larvae per stalk in 1981 to a low of 0.7 per stalk in 1994. Stem weevil numbers were extremely low again in 1995 with only a mean of 0.2 larvae recovered per stalk (unpublished data).

**Biology and Population Dynamics**

Adult sunflower stem weevils emerge from overwintered stalks and root crowns in early to mid-April in the southern Plains and mid-to-late June in the northern Plains. Females deposit eggs under the epidermis at the base of sunflower stalks (Rogers and Serda, 1982; Charlet, 1987b). Adults feed on stem and leaf tissue. Adults are present in the fields in the northern Plains until late August with peak densities in mid-July (Charlet, 1987b). Eggs are initially deposited around the first node (cotyledon) and the height of egg placement in the stalk increases over time. Higher numbers of adult weevils, probably due to competition for sites, increases the height that eggs are laid. In North Dakota, 50% of eggs are deposited by mid-July (Charlet, 1983c). Under laboratory conditions, females oviposited 0.5 to 5 eggs per day for a total production of 24-195 eggs, depending on temperature. The greatest number of eggs was deposited at 30°C. Females survived up to 75 days when held at 20 or 23°C (Barker, 1987). Larvae hatch in early July and early instars feed in the vascular tissue and, as the larvae mature, tunnel into the pith. Larvae feed apically until early August and then descend to the lower portion of the stalk or root crown by late August and excavate overwintering chambers by chewing cavities into the stem cortex (Rogers and Serda, 1982; Charlet 1983a). There is only one generation per year (Charlet, 1987b).

Feeding by adults on the stem and leaf tissue causes minor damage to the plant. If the larval population in a plant is high, the stem, weakened by tunnelling, pith destruction, or overwintering chambers, will break causing a loss of the entire capitula prior to harvest. In North Dakota, a mean infestation of 38 larvae resulted in 28% lodging (Charlet et al. 1985). Adult numbers high enough to require chemical treatment were detected in Stutsman County in 1989 and reports of lodging exceeding 50% of sunflower fields were received from McHenry and Bottineau Counties. Aerial applicators reported in a survey taken by the North Dakota Department of Agriculture that over 24,000 acres were treated for adult sunflower stem weevil in 1989 in south-central North Dakota (Charlet 1991). Armstrong (1996) reported severe damage from weevils in eastern Colorado and western Kansas and Nebraska in 1993 and 1994 with over 80% pre-harvest lodging in some areas.

Stalk breakage due to the sunflower stem weevil is most severe during drought stress or when high winds occur as plants are drying prior to harvest (Charlet, 1991). Populations of over 80 larvae/stalk in irrigated sunflower in the southern Plains were required to cause a yield loss from larval feeding (Rogers and Jones, 1979). The sunflower stem weevil has also been implicated in the epidemiology of sunflower pathogens, such as Phoma black stem (*Phoma macdonaldii* Boerma), that contribute to the premature ripening syndrome in the northern Plains and may predispose plants to infection by *Macrophomina phaseolina* (Tassi) Goid, the causative agent of charcoal stem rot in sunflower in the southern Plains. Phoma has been implicated as one of the major biotic causes of premature ripening syndrome (early dry down) of sunflower in North Dakota. Although premature ripening is probably caused by a combination of both abiotic and biotic factors, evidence shows that stem-infesting

insects may transmit disease organisms or encourage the disease by serving as a plant-stressor. (Gaudet and Schulz 1981, Yang and Owen 1982, Charlet and Gulya 1984, Gulya and Charlet 1984). Lodging is a good indicator of larval densities; however, lodging is also influenced by other factors including stalk diameter, cortex and pith thickness of the stem, weight of sunflower heads, wind velocity and direction, position of larvae in overwintering chambers in the stalk, and incidence of disease (Charlet 1991).

Studies conducted in Texas between 1978 and 1980 showed that adult sunflower stem weevil emergence began late April to early May and ended by mid-June (Rogers and Serda 1982). In North Dakota adult emergence started between 6-11 June and ended 16-25 July between 1982 and 1984 (Charlet 1987a). In 1995 research in Colorado showed that adults began emerging from stalks 6 June and emergence ended on 11 July (Armstrong 1996). Degree-day (DD) models have been developed to predict emergence. Charlet (1987a), using a base temperature of 5°C, determined that first emergence occurred at 420 DD and by 865 DD 90% of adults had emerged. Armstrong (1996) reported first emergence at 379 DD and 90% emergence at 651 for populations in Colorado also using a base temperature of 5°C. Studies conducted by Barker and Charlet (1993) showed that 12°C was the threshold temperature for post-diapause development of sunflower stem weevil in the laboratory with 365 DD required for adult eclosion. An additional 2-3 days were needed before adults achieved mobility.

Larvae of the sunflower stem weevil overwinter as mature larvae in chambers constructed in the lower stalk or root crown of the sunflower plant (Rogers and Serda 1982, Charlet 1987b). Rojas et al. (1994) reported that larvae survive through their avoidance of freezing by a supercooling capacity derived from accumulating high levels of trehalose. Larvae pupate in these chambers in the spring in infested stalks, from which adults emerge by chewing through the stalk epidermis (Rogers and Serda 1982). Charlet (1989) noted that overwintering survival of the larvae varied with microhabitat and that the mortality of larvae was not increased unless larvae were exposed in the soil. No difference was detected between survival of weevils overwintering in exposed or buried stalks (Charlet 1989).

Rearing procedures have been developed to culture the sunflower stem weevil using a laboratory produced non-diapausing strain. The host plant is required for feeding and to induce oviposition in the weevils. Approximately 40-50% of the eggs are laid at random (not inserted into the sunflower stem). Eggs are placed on a modified boll weevil diet to rear the larvae. Approximately 42% of the eggs develop into larvae and 80-90% of the larvae develop into adults (Barker 1989, Barker et al. 1989).

**Management Strategies**

Effective insect pest management requires a broad approach that incorporates knowledge of the insect's biology and population dynamics, determination of economic injury levels, the use of resistant cultivars, as well as biological, cultural, and chemical controls. The ideal control strategy utilizes techniques that require low input costs, are cost-effective, and avoid negative impacts on the environment. Management techniques that reduce weevil densities in the stalks or improve the plant's ability to tolerate weevil attack should be utilized.

Monitoring for sunflower stem weevils is important in determining if densities are present that could result in economic losses to the field. However, adults are difficult to locate on the plants due to their small size and behavior. They are inactive on the plant or fall to the ground when disturbed and remain motionless. Adults can be found on both surfaces of the leaves, the lower portions of the stem, in leaf axils, within the dried cotyledons, or in soil cracks at the base of the sunflower plant. Sampling for the larval stage is difficult since they develop totally within the sunflower plant. Thus, the only method for detecting the presence of larvae is to split the sunflower stem, a time-consuming process. Studies have shown that average counts of 1 adult sunflower stem weevil per 3 plants (based on sampling during the 2-week period between 24 June and 7 July) resulted in stalk densities of over 40 larvae per stalk at the end of the season (Charlet 1987b). Levels of 38 larvae per stalk resulted in 28% lodging of sunflower plants (Charlet et al. 1985). Control strategies directed at the adults must be initiated prior to mid-July, by which time 50% of egg deposition has occurred. Since damage results from lodging of larval infested stalks, anything that promotes thick healthy stems helps to reduce losses. Even with the same number of larvae, a plant that has stems of increased diameter and greater stem density will be less likely to break. Therefore, lower plant populations, adequate fertilization, and proper soil moisture should aid in decreasing sunflower lodging (Charlet 1991).

**Cultural Control.** Cultural control tactics use farming practices associated with crop production. They are effective because they make the environment less favorable to the pest or more favorable for the plant. Cultural control tactics include the following approaches: planting date, tillage, crop spacing or plant population, trap crops, crop sanitation, and crop rotation. Cultural controls have the advantage of usually requiring no additional outlay for equipment, lack deleterious side-effects and are generally simple, effective, and inexpensive to apply. However, cultural control measures often need to be planned in advance, the control of the pest is not always complete, and a knowledge of the pest's biology is required.

Delayed planting was effective in both the northern and southern Plains as a management tool in lowering larval densities in sunflower stalks (Rogers and Jones 1979, Oseto et al. 1982, Rogers et al. 1983, Charlet and Brewer 1994). Stem diameter is an important factor determining the ability of the sunflower plant to withstand lodging of weevil infested stalks. Although research in North Dakota in 1990 and 1991 showed that the stem diameter decreased with later planting dates, the density of larvae also decreased significantly, thus reducing the potential for lodging. Sunflower stem weevil larval numbers decreased from 21 to 2 and from 23 to 2 larvae per stalk when planting dates were delayed from mid-May to early June at Carrington and Prosper, North Dakota, respectively (Charlet and Brewer 1994).

Plant population impacts both the diameter of sunflower stalks and percentage of lodged plants. Larval stalk population (average of 12 larvae per stalks) was not affected by the plant density within the plots. However, stalk diameter was significantly different among all three plant populations, with the thinnest stalks in the most dense plantings. Lodging was low at both the 22,000 and 45,000 plants per hectare plots. However, almost 25% of the plants were lodged when the density of sunflower stalks increased to 89,000 plants per hectare. Larvae overwinter in stalks by constructing chambers in the stem cortex. These chambers weaken the plant and if larval numbers are high, the plant can lodge prior to harvest with a

loss of the entire head. Maintaining the internal structural integrity of the sunflower stalk apparently prevents lodging of the plant. Results from North Dakota showed that with no change in insect levels in the stalk, reducing the plant population density can result in decreased damage from lodging (Charlet and Brewer 1994, Charlet 1996).

Cultivation of crop residues has been shown to be another effective cultural control method with some crops. The sunflower stem weevil overwinters as a mature larva in chambers constructed by the larva in the lower stalk or root crown. Larvae pupate within the overwintering chamber and adults then chew through the stalk epidermis and exit from the stalk (Rogers and Jones 1979). A study conducted by Charlet (1994a) showed that stalk burial severely impacted emergence of adult stem weevils. Mortality may have been due to the inability of adults to move from the stalk through the soil to the surface. Earlier studies discounted the impact of tillage as a control strategy for the sunflower stem weevil. Charlet (1989) noted that larvae seemed to be protected within stalks, and unless the stalks were broken and the larvae exposed in the soil, survival would not be significantly decreased by plowing. Rogers et al. (1983) found that fall and winter disking or sweep plowing did not affect weevil mortality. Protection of the larvae in the woody portions of the stalk prevented physical injury of the larvae from the implements. However, it appears that burying stalks at least 15 cm below the soil surface may decrease populations of the stem weevil the following season by reducing adult emergence (Charlet 1994a). Thus, a combination of disking to break up the stalks and the use of moldboard plowing to bury the stalks and seal the soil could provide excellent control in areas that have experienced damaging populations of the sunflower stem weevil. However, because sunflower is rotated each year and infestation is based on migrating weevils, single field treatments might not protect a specific field. Only area-wide tillage would impact the number of weevils migrating into each season's new sunflower fields. In addition, the value of standing stalks in holding snowfall to insure adequate moisture in the field and impact of plowing on soil erosion must also be considered.

**Chemical Control.** The use of both foliar and systemically applied insecticides has been shown to be effective in reducing larval populations and percentage of stalks lodged (Charlet and Oseto 1983, Rogers et al. 1983, Charlet et al. 1985). However, chemically reduced larval populations have not consistently resulted in greater seed yields. In the northern Plains, treatment must be initiated from late June to early July before appreciable egg laying has occurred to reduce larval numbers in the stalk (Charlet, 1987b). Although earlier research has shown that chemical control can be effective in reducing densities of weevil larvae in the stalk and the percentage of plants lodged (Charlet and Oseto 1983, Charlet et al. 1985), insecticides also destroy natural enemies of the weevil and may contaminate the environment. In addition, the number of registered insecticides available to the producer has been reduced and may decline further in the future.

**Plant Resistance.** Host-plant resistance is a pest management method that utilizes the plant's own defense mechanisms against the insect to either avoid attack, destroy the insect, or tolerate the injury. The resistance is developed through plant screening and breeding. An important advantage of this strategy is that pest-resistant cultivars, once produced, become a cost-effective and environmentally safe form of insect management and are usually compatible with other pest management approaches in sustainable agriculture. Evaluation of

the plant resistance for adverse effects on the natural enemies of the pest is also important. Greenhouse and field experiments have shown resistance to feeding, oviposition, and larval development in many native species of sunflower (Rogers and Seiler, 1985). Barker (1991) indicated that although there was reduced feeding by adults on some native sunflower species, it was not associated with high trichome density. Research into the development of resistant sunflower has been hampered by inconsistent field populations for screening of potentially useful germplasm and thus can only be conducted when field densities are high. Comparison of numbers of weevil larvae in stalks from both Prosper and Carrington, North Dakota, between 1989 and 1991 showed significant differences among different sunflower lines. Numbers of larvae varied from 4 to 59 per stalk showing that some lines have potential resistance to infestation by the stem weevil. *Nealiolus curculionis* (Fitch) (Hymenoptera: Braconidae) parasitization rates also varied among lines, with high parasitism in some lines that had low weevil numbers. One accession had only 9 larvae per stalk, but a 34% parasitism rate. Parasitism also varied among some lines with similar weevil densities. Some lines were very susceptible to attack by the sunflower stem weevil and also seemed to have a detrimental effect on the parasitoids of the weevil (average density of 25 larvae per stalk with less than 9 percent parasitism). The mechanisms that influence parasitism of weevil larvae among different sunflower lines need to be further investigated (Charlet and Brewer 1992, Charlet 1996).

**Biological Control.** The biological control of pests by natural enemies includes importation and establishment of exotic species, augmentation of established species, and conservation of species through manipulation of the environment. The conservation of natural enemies in a crop agroecosystem is important in maintaining pests below levels that cause economic damage. The naturally occurring or indigenous natural enemies prevent many plant-feeding insects from achieving pest status. The conservation of these natural enemies allows them to operate at their full potential. Manipulating the environment to eliminate or mitigate adverse factors, such as pesticides, can effectively conserve the natural enemies present. Conservation of natural enemies depends on understanding the agroecosystem, the use of selective pesticides, the use of the least disruptive formulation of the chemical, application of insecticides only when necessary and based on sound economic injury levels of the pest, and pesticide application at the least injurious time or place.

The sunflower stem weevil is attacked by both egg and larval parasitoids. The eggs of the weevil are attacked by *Anaphes pallipes* (Ashmead) (Hymenoptera: Mymaridae) (Charlet and Balsbaugh 1984). Hymenoptera recovered from overwintering larvae include: *Tetrastichus ainsliei* Gahan (Eulophidae); *Mesopolobus* sp. (Pteromalidae); and *Nealiolus curculionis* (Charlet 1983d). The latter species is the most prominent of the larval parasitoids attacking the weevil. *Nealiolus curculionis* is a univoltine, solitary, endophagous larval parasitoid of the sunflower stem weevil in both cultivated and native sunflower. This parasitoid represented 96% of all parasitoids attacking the weevil in studies conducted from 1980 to 1991. Adult parasitoids are active in the field from late June to late August. Eggs are deposited in early instar weevils feeding within the sunflower stalk. The immature parasitoids overwinter within diapausing weevil larvae in the sunflower stalk (Charlet 1994b). Results indicated that overall parasitization increased from levels reported in the late 1970's and early 1980's. Parasitism of stem weevil larvae by *N. curculionis* has averaged 27% since

1983, while stem weevil densities have varied from 6 to 29 larvae per stalk. The consistent rates of parasitism compared with the variable field densities of adult parasitoids suggest that *N. curculionis* effectively forages for hosts under varying host population densities. This parasitoid appears to be a consistent mortality factor in the population dynamics of the sunflower stem weevil in cultivated sunflower (Charlet, 1994b). Rogers (1980) and Rogers and Serda (1982) reported two parasitoid species attacking the sunflower stem weevil in Texas: *N. curculionis* and *Neocatolaccus tylodermae* (Ashmead) (Pteromalidae). *Rhaconotus cressoni* Muesebeck and Walkley (Braconidae), *Eupelmus cushmani* (Crawford), *E. cyaniceps* (Ashmead) (Eupelmidae), and *Zatropis incertus* (Ashmead) (Pteromalidae) have also been reported to parasitize larvae of the weevil (Casals-Bustos 1976, Krombein et al. 1979). The impact of pesticides on natural enemies must be considered in developing management programs, since insecticides that are toxic to the weevil also reduced parasitism (Charlet and Oseto 1983).

**Summary and Future Needs**

Current knowledge includes information on the biology and population dynamics of the sunflower stem weevil. Economic injury levels have been described and a number of management strategies are available to reduce larval densities and losses from plant lodging. Additional studies are needed to determine adult distribution patterns in fields and sampling procedures to make population assessment faster and more reliable. Since lodging is dependent on stalk structure and strength, the economic injury levels need to be refined to include this type of data. Future studies should also continue the search for germplasm with resistance to the sunflower stem weevil. We also need a better and less time consuming method to determine larval numbers within the stalk for damage assessment. A new method could be used to compare germplasm for resistance and evaluate the efficacy of other management strategies in reducing sunflower stem weevil populations and damage. Additional research should also be conducted to locate new natural enemies, including pathogens and parasitoids, or improve their impact in managing the sunflower stem weevil.

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# The biology and management of the sunflower seed weevils, *Smicronyx fulvus* and *S. sordidus:* Past, Present and Future

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## Historical Perspective

Sunflower seed weevils are major insect pests of sunflower in North America (Charlet et al. 1987) and have been pests since modern varieties were first planted (Cockerell 1915, 1917, Forbes 1915). Seed weevils are found throughout the Great Plains from Canada to Texas. In the northern plains the red sunflower seed weevil (rssw), *Smicronyx fulvus* LeConte (Coleoptera: Curculionidae), is more common, in the southern plains the gray sunflower seed weevil (gssw), *S. sordidus* LeConte, is predominant although the species overlap.

## Life Cycle

Although both species occur together on sunflower heads (capitulum), oviposit on floral tissues or seeds, and are internal seed feeders as larvae; they differ in plant stage preference and choice of an oviposition site (Brewer 1991). The gssw arrives first, at the bud stage, and oviposits at stages R3 through R4. Typically, eggs are placed in the tips of unopened florets. Oviposition ceases with pollen shed and the adult weevils move to other heads still in the bud stage. The rssw prefers heads shedding pollen (R5) and places eggs between the pericarp and kernel of the developing seeds. Adult rssw remain on the plant through pollen shed. Rssw females need to feed on sunflower pollen or other tissue to mature eggs (Korman 1984, Rana 1995) but gssw do not seem to need a preoviposition period and a specific nutrient source to mature their eggs. Seeds infested by gssw enlarge and become completely hollow. A seed damaged by larval rssw feeding has only part of its kernel missing and is of normal size (Brewer 1991).

In the fall, larvae of both species drop to the soil to overwinter. In June of the following year they begin to pupate and adult rssw emerge in July. Gssw adults emerge several weeks earlier. Oviposition begins as plants develop to the preferred growth stage. Larvae feed and develop internally in the seeds until late summer or fall.

## Experimental Methods and Rearing

Brewer (1991) used single-plant cages to enclose single sunflower heads and to confine rssw or gssw to the heads for artificial infestation. The initial cage design used a frame to keep the cage walls away from the head. Subsequently it was found that the framework was not needed and that a Delnet pollination bag (Applied Extrusion Technologies, Middletown, Del.) was sufficient to enclose the weevils. Because the rssw only oviposits in developing seeds, allowing cross-pollination to occur in self-incompatible lines before caging the plants is important.

Methods to rear field collected larvae to the adult stage were developed by Brewer (1992).

**Seed Weevil Management**

Since 1974, the rssw has been an economically important pest of sunflower production in North Dakota (Oseto and Braness 1979). If populations are sufficiently high, yield and oil content are economically lowered (Peng and Brewer 1995a).

Seed weevil management in oilseed sunflower has focused on the rssw. The rssw has been a consistent pest in the Dakotas and Minnesota, the main sunflower growing region in the United States. In most regions where sunflower is grown the rssw is more abundant than the gssw and is more prolific than the gssw. As a result, populations of the gssw are normally too low to cause economic damage and controls directed against the gssw are not needed.

Because confection sunflower with more than 3 to 4% insect damaged seed is rejected, a lower level of damage causes loss and the likelihood of the gssw being economically important is increased. Nevertheless, even in confection sunflower, the gssw may not be economically important because seeds infested by gssw enlarge, have brittle hulls, and are hollow (Stober 1993). The threshing and seed cleaning processes probably remove most gssw damaged seed. So even in confection sunflower, the likelihood of seed being rejected because of gssw damage is low.

If the gssw is suspected of being economically important, sampling and control decisions need to be taken earlier than for the rssw. Because the gssw oviposits on sunflower in the bud stage and chemical controls are directed against the adult to prevent oviposition, sampling needs to begin at bud stage R3 or earlier. If the decision is made to apply insecticides, they would have to be timed to prevent gssw oviposition. As the plants begin blooming, separate sampling and control decisions would need to be made for the rssw.

## RSSW MANAGEMENT YESTERDAY

Early rssw management research focused on cultural and chemical controls. Oseto et al. (1987) showed that early planting reduces rssw damage and in most years did not significantly reduce seed weight or oil content. Early planting allows sunflower to pass its most susceptible stage (mid to late bloom) before the highest rssw populations occur. However, because early planting favors the development of economically damaging populations of the sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte) ( Oseto et al. 1982) and banded sunflower moth, *Cochylis hospes* Walsingham (Oseto et al. 1989), planting date decisions should consider the potential harm from all species.

Tillage can also affect rssw populations. Fall or spring moldboard plowing can reduce rssw adult emergence by up to 56% (Gednalske and Walgenbach 1982, 1984a). But because the rssw migrates to new fields each season, tillage operations to manage the rssw would have to be done on an area wide basis to be effective.

Gednalske and Walgenbach (1984b) applied insecticides at early bloom and again three days later. The insecticides significantly reduced the amount of damaged seed but the effect of application time was inconsistent. In another study of the effect of insecticide timing on rssw control, treatments made when most of the plants were in the R5.1 to R5.4 stages of anthesis gave the best control (Oseto and Burr 1990).

## RSSW MANAGEMENT TODAY

Recently trap cropping, sampling methods, and the economic injury level have been evaluated or revised. Trap cropping was tested in commercial oilseed sunflower fields ranging in size from 22 to 60 ha (Brewer and Schmidt 1995). They found that the yield for trap crops was comparable to conventional (whole-field) insecticide treatment. However, in a trap crop only the trap rows (the field margin comprising about 10% of the field area) are treated. So the cost of control is much less than whole-field treatment. The result is equivalent yields while saving about 90% of the cost of insect control.

Rssw adults have an aggregated distribution in sunflower fields with plant stage affecting population densities. Blooming plants were preferred to bud stage plants and stage R5.4 to 6.0 was preferred to stage R5.0 to R5.3 (Peng and Brewer 1994). The distribution of damaged seeds is also aggregated and a fixed size sample plan is given by Peng and Brewer (1995b) to sample for damaged seeds. Two studies looked at the distributions of damaged seeds in a field. Charlet and Oseto (1982) sampled fields for damaged seeds at the margin (0), 7 and 15 m and reported that the number of damaged seeds was higher on the field margin. Peng and Brewer (1995b) began sampling 10 m in from the field margins and sampled for damaged seeds throughout the fields. Damaged seeds were evenly distributed in their study. To avoid the edge effect detected by Charlet and Oseto (1982), begin sampling for damaged seeds at least 10 m from the field margin.

Insecticides are used to reduce seed weevil damage to sunflower by limiting oviposition and are directed against adults (Peng and Brewer 1996). Rssw eggs and larvae are inside developing seeds (Brewer 1991) and are not accessible to chemical controls. Economic thresholds (ET) are used to compare the potential of insect populations to cause economic loss against the cost of controls. If the potential loss is equal to or greater than the cost of control, insecticide use is recommended. Oseto and Braness (1980) developed an ET for the rssw. However their ET did not consider oil content loss in damaged seeds, was based on fecundity studies of caged weevils, and the relationship between adult counts at different plant stages and the number of damaged seeds was not defined. Peng and Brewer (1995a) revised the ET. The revised ET includes the oil content loss in damaged seeds and is based on samples taken at plant stages R5.0 to R5.3. For a plant population of 45,000 per ha and a market price of $0.15/kg the revised ET is 8.9 weevils per plant; the equivalent ET using the Oseto and Braness (1980) formula would be 26.8 weevils per plant.

An efficient and reliable sampling method is needed to make accurate treatment decisions based on the ET. A fixed sample size plan requiring 25 samples has been used to estimate rssw population levels (McBride et al. 1992). Usually sequential sampling plans are more efficient because they need only 40 to 60% as many samples as fixed sample size plans. Peng and Brewer

(1996) developed a sequential sampling plan for the rssw. The number of samples needed is indeterminate. At population levels considerably below or above the ET, the sequential sampling plan requires fewer samples than the fixed plan. At population levels near the ET, the sequential sampling plan requires more samples than the fixed plan, suggesting that the fixed plan does not give adequate precision.

To use the sequential plan, begin sampling 10 m in from the field edge when most plants are at stage R5.1 to R5.3. Because weevils are evenly distributed throughout the field, the number of sample sites is not critical but because of the strong preference of weevils for plant stage, plants should be randomly selected and representative of the mix of plant stages in the field. If the total weevil count after each sample is below the lower stop line, sampling stops and no control action is recommended. When the count is above the upper stop line, the ET is met or exceeded and chemical controls should be initiated. If the count is between the stop lines continue sampling. If no decision is made before 55 (ET of 6) or 75 (ET of 8) samples are taken, calculate the sample mean. If the sample mean is larger than the ET, treat the field.

The sampling plan is based on absolute weevil counts. In-field counts miss some weevils and underestimate population levels. If sunflower heads are sprayed with mosquito repellent containing diethyl toluamide, the weevils will drop from the head. Weiss and Brewer (1988) give a method of converting in-field counts to absolute counts using the repellent method .

## RSSW MANAGEMENT TOMORROW

Research efforts that will affect rssw management in the future are biologically based, low-impact, low-input methods. The importance of chemical control should lessen as plant resistance and biological controls become available.

Brewer and Charlet (1995) and Brewer et al. (1995) examined resistance to rssw in *Helianthus annuus*. Charlet and Seiler (1994) found indications of rssw resistance in several native *Helianthus* species. Other research is underway to produce transgenic sunflower with resistance to the rssw (Grayburn 1994, 1995). These approaches should result in rssw resistant sunflower in the future. Efforts to develop transgenic sunflower are based on a microbial toxin produced by *Bacillus thuringiensis*. Traditionally *B. thuringiensis* has been used as a microbial insecticide and an offshoot of the development of transgenic sunflower may be a *B. thuringiensis* product that can be used as a conventional pesticide.

Charlet and Seiler (1994) found five species of endoparasitic Hymenoptera parasitizing weevil larvae from eight native *Helianthus* species. *Urosigalphus femoratus* Crawford (Braconidae) was the most prevalent species. Rssw parasitizism rates have been increasing (4 to 50%) in cultivated sunflower from 1988 to 1993 (Charlet 1994). Roehrdanz et al. (1996) are developing methods to use molecular markers to identify parasitoids attacking sunflower insects. These will aid in the correct identification of this taxonomically difficult group. Entomopathogenic nematodes are another potential biological control agent. Both *Steinernema feltia* and *S. carpocapsae* can infect and kill rssw larvae in the lab (Wozniak 1994).

## Conclusions

Of the two species of seed weevils, the rssw is a consistent economic pest. Chemical controls have given good results and a revised ET and an improved sampling procedure should make their use more efficient. In the future, reliance on chemical controls may lessen as practices such as trap cropping are adopted and as plant resistance and biological controls become available.

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# The Sunflower Moth

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## Distribution of the Pest

The range of sunflower moth is from Mexico and Cuba to Canada. It is usually found in all regions where wild or domesticated sunflowers (*Helianthus*) grow.

## Fecundity

There is some variation in researchers reports of egg counts from the moth. One said "a single female laid 91 eggs, mostly in a single day". Another said "females lay 30 or more eggs either singly or in groups of 4-5 within or among corolla tubes of individual florets". Also "most oviposition takes place on the 3rd day after the head opens and 75% of eggs are laid by 6th day" and "lab moths laid 337 eggs in a 2 week period". In our 1986 study at Ames, IA, we observed that 107 females laid 11,100 eggs in 8 days for an average of 103.7 eggs/female.

## Emergence Period

Adults have been observed in N. Dakota and Canada from early to mid-July. They migrate from the south central U.S. Published records note first observations in the following states: Iowa (early July), Missouri (late June), Georgia (July), Texas (late April), California (early June), and Mexico (November to April).

## Life Cycle and Seasonal History

The basic life cycle of the sunflower moth is as follows: Egg - hatch within 3-5 days; Larva (4-5 instars) with the 1st being $\leq$ 4 days, the 2nd = 3-5 days, the 3rd = 5 days, the 4th = 1-3 days, and the 5th = 10-12 days; Pupa pupate in soil for 6-7 days, and the Adult lives a couple of weeks.

Diapause depends on both temperature and photoperiod and is induced more readily at 21° C with < 10 hrs light. Diapausing prepupae cannot survive -5° C for 7 or more consecutive days so are absent north of roughly 40° N. latitude. Nondiapausing larvae died after 1 day at -10° C but 33% of diapausing larvae survived to pupate after 7 days at -10° C. This suggests that SM is capable of overwintering much farther north than was previously thought.

## Description of Life Stages

Egg: pearly white, elliptical, finely reticulated, from 0.63-0.80 mm long and 0.23-0.27 mm diameter; Larva: brown head capsule, purplish or reddish brown body with alternate dark and light stripes running longitudinally; from 19-25 mm long; Pupa: reddish-yellow to brown, 10 mm long; Adult: buff to gray moth with wing span of 19-20 mm and body length of 9-11 mm.

## Damage and Pest Stages Involved

Pest damage is described in the literature as follows: "larvae laid a delicate silk over surface giving a trashy appearance - early instars feed on florets rather than achenes", "a single larva damaged 9 achenes in a 3 week period", "single larva feeds on 3-12 seeds", "severe infestations can cause 30-60% loss", and "a

two year study found sunflower moth larva consumed 0.29g and 0.23 g seed, respectively".

**Economic Injury Levels - Economic Thresholds**

Researchers have found that "From 12 - 24 larvae per head causes serious seed loss" and "the economic threshold is 1 to 2 adults per 5 plants at onset of bloom".

**Field Sampling/ Monitoring**

The field is sampled using an X - Pattern, beginning at least 75-100 ft from the field margins. Sunflower moths are counted on 20 heads per sample. Five samples are taken for a total of 100 heads per field.

**Pheromones or attractants**

Primary sex pheromone is Z-9, E-12-tetradecadien-1-ol (Z9,E12-14:OH). Also Z-9-tetradecen-1-ol (Z9-14:OH) may be important when acting in combination with the primary sex pheromone.

In the interaction of sunflower moths with the sunflower plant, there appear to be important chemicals present in the pollen. An ethanol extract of pollen was shown to be an oviposition stimulant. Presence of pollen caused females to initiate calling behavior at a younger age and they spent more time calling. This resulted in an increased rate of egg maturation.

**Relationships with Diseases**

Few diseases have been identified that attack sunflower moth. A nosema fungi has been identified that attacks the European sunflower moth. This fungus attacks other Pyralids (e.g., European corn borer) so it could potentially be a control method for the sunflower moth. In Missouri, researchers found a fungus on sunflower moth called *Metarrhizum anisopliae.* They didn't mention whether or not this would be a useful "potential" control agent.

**Rearing in the Laboratory**

The rearing diet began with the basic wheat germ diet developed by Adkisson et al. and later modified by Vanderzant. Rogers modified the diet by substituting B-vitamin (with inositol) and ascorbic acid for vitamin mix. He added two anti-fungal compounds (potassium sorbate and methyl p-hydroxybenzoate). Wilson modified the diet by using vitamin fortification mix (ICN National Biochemicals) and also added an antifungal compound. He also used a microwave oven for heating agar which helps prevent burning the agar during the cooking process.

Our rearing procedure starts with one gallon size oviposition jars in which the bottoms are covered with about 5 cm of fine white sand. The sand is lightly dampened with water and covered with 15 cm filter paper. A 30 ml cup with a cotton wick and 5% sucrose solution is added to feed the adults. Oviposition pads (8 $cm^2$) are suspended inside jar. The rearing room is kept at 24-27° C, with 60% RH, and 14:10 (light:dark). About 250 pupae, hand picked from the diet 2-3 days after pupation, are placed in a 9 cm petri dish and placed into the adult oviposition container.

Egg are collected by lifting the oviposition pad gently from jar, and shaking off any moths that may cling. Most eggs are obtained from day 3-5. Wilson and McClurg found the eggs can be stored in 5° C for up to one week without significantly reducing egg hatch. When we need larvae for testing, the eggs are moved to a 27° room where they will hatch in 2-4 days. The larvae are reared in either a 26 cm diameter (large) or 30 ml capacity (small) plastic container. The diet is scored (scratched with a sharp object) before introducing larvae. We place about 600 larvae in the large containers, but the small containers hold 6-8. It takes about 3 weeks for late instar pupae to develop.

**Alternate Hosts**

The sunflower moth has been found on many plant families but mostly on the Compositae (Asteraceae). The list includes, but is not all inclusive, of some of the following plants: Rosering gaillardia, Golden crownhead, Goldenmane, Lanceleaf gaillardia, Englemann daisy, Twoleaf senna, Big flower, Gumweed, African marigold, French marigold, Golden wave, Tickseed, Orange coneflower, Yellow chamomile, Romerillo blance, Citrus, Safflower, Sweetclover, Corn, Globemallow, Musk thistle, and many species of *Helianthus*.

**Management - Using Natural Enemies**

Beregovoy recovered 17 species of Hymenoptera and Diptera parasites from sunflower moth larvae. In a Missouri study, they found 12 parasites: *Lixophaga variabilis, Erynnia tortricis, Leskiomima tenera, Clausicella floridensis, Bracon mellitor, Bracon nuperus, Chelonus altitudinus, Apanteles homoeosomae, Agathis buttricki, Macrocentrus ancylivorus, Creamastus epagoges,* and *Perilampus epagoges.* Three were found in a Texas study: *Chelonus altitudinus, Apanteles epinotiae,* and *Clausicella neomexicana*. In California, four were found: *Mesotimes gracilis, Pristomerus pacificus, Apanteles homoeosomae,* and *Euxesta anna*. And in North Dakota two others were listed: *Lixophaga plumbea,* and *Erymnia tortricis.* Some of the parasites are found in more than one location.

**Management - Host Plant Resistance**

Searching for plant resistance in 1966, Kinman found a single sunflower plant with 10% damage and it was incorporated into the pedigree of hybrid T56002. In 1971, Teetes et al. showed differences in susceptibility to selected varieties. The Russian varieties ‘armavirec’ and ‘kubanec’ were less damaged than was inbred HA 6. Carlson and Witt identified resistance and tolerance in cultivars H2131 and H2135 and others in 1974. And in 1980, Jarvis evaluated 350 National Plant Germplasm System sunflowers at Ames, IA and found three resistant *H. annuus* accessions (PI 172906, PI 204578, PI 380569).

In 1984, Seiler et al. looked at 50 species of wild sunflower and found the resistance factor, phytomelanin, in pericarp of them all. Then in 1993, Dozet et al. tested populations of 23 species for presence of phytomelanin layer. The species with the highest percent phytomelanin layers include: *Helianthus salicifolius* (85%), *H. laevigatus* (80.5%), *H. strumosus* (73%), *H. resinosus* (75%), *H. giganteus* (67.5%), and *H. grosseserratus* (66%).

The literature cites other possible sources of sunflower moth resistance: *H. ciliaris, H. decapetalus, H. maximiliani, H. occidentalis ssp. occidentalis, H. pumilus, H. silphioides, H. strumosus, H. tuberosus,* and *H. petiolaris ssp. petiolaris.*

Some biochemical compounds are involved in host-plant resistance of

sunflower to the sunflower moth. In 1985, Gershenzon et al. found high levels of sesquiterpene lactones and diterpenes in glandular hairs on wild species of *Helianthus*. Rogers et al. tested 30 perennial and 11 annual species of *Helianthus* in 1989 and noted that plants were resistant when terpenes were found in floral parts and phytomelanin in seed pericarp.

At the North Central Regional Plant Introduction Station, we evaluate domesticated sunflower for sunflower moth resistance by the following technique: (1) select test accessions from the sunflower collection and include the checks '894' and 'Arrowhead', (2) plant each accession in 2 row plots, 25 ft long, (3) infest the accession when 20 plants are at the R5.2 stage, (4) 10 plants are labeled and infested with sunflower moth eggs, (5) another 10 plants are sprayed with Asana® after one week, (6) 1-2 days after spraying, all 20 plants are covered with muslin bags. By waiting one week, this allows natural pollination to occur before bagging. (7) the heads are harvested at physiological maturity, (8) head diameters are measured, the seed are removed by hand, and the seed are cleaned, weighed and counted, (9) gm seed/$cm^2$ and no. seed/$cm^2$ are calculated, (10) % of control is calculated by dividing infested head data by check head data, (11) accessions with % of control values$\geq$ 100 are considered resistant.

Some results from 1994: 48 accessions were tested, 8 had both % of control values for no. seed/$cm^2$ and gm seed/$cm^2$ > 100%. The resistant accessions are: PI 162453, PI 170389, PI 170401, PI 170420, PI 170428, PI 176975, PI 250853, and PI 301060.

Released germplasm: In 1984, Rogers et al. released 3 germplasm lines for resistance to sunflower moth; SFM 1 is an interspecific cross of PI 181954 X PI 423011 [H. petiolaris], SFM 3 is a cross of PI 181956 X PI 423011 and SFM 2 is a cross of PI 356301 X HA 89. All of the germplasm lines have genes that encode production of phytomelanin in their pericarp.

**Management - Cultural Control**

Planting date studies in Nebraska, Texas, and Georgia showed that early planted sunflowers had smaller infestations of sunflower moth. A Kansas study found that delaying planting until the middle of June reduced sunflower moth infestations and with no significant loss in yield.

**Management - Chemical Control**

The currently registered chemicals for control of sunflower moth are:

Asana® (esfenvalerate)
Sevin® (carbaryl)
Furadan® (carbofuran)
Lorsban® (chlorpyrifos)
Thiodan® (endosulfan)
Helena/Setre® (methyl parathion)
Supracide® (methidathion)
Dipel® (BT)

**Miscellaneous**

Some possible areas to investigate: (1) answer the question: How far north can the sunflower moth overwinter?, (2) find and develop cultivars with pollen and/or floral toxicants, (3) understand better the chemistry of the phytomelanin

layer - develop a quick test to quantify amount present, (4) continue to examine other species of *Helianthus* for sources of sunflower moth resistance, (5) identify chemicals affecting behavior and calling in sunflower moth, (6) identify chemicals in pollen that stimulate oviposition, (7) locate disease organisms that could be used for control, (8) utilize parasites for control, and (9) conduct IPM studies that involve several control tactics in combination.

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# The Biology and Management of the Sunflower Beetle, *Zygogramma exclamationis:* Past, Present and Future.

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## Introduction

The sunflower beetle, *Zygogramma exclamationis* (Fabricius)(Coleoptera: Chrysomelidae) has been a prominent pest in commercial sunflower in the northern Great Plains (McBride and Charlet 1985), the Prairie Provinces of Canada, especially Manitoba (Criddle 1922, Westdal and Barrett, 1955), the central Great Plains (Walker 1936, Rogers 1977, and Powell 1932) and has been present but without pest status in the southern Great Plains, especially in the northwest one-third of Texas (Rogers 1977). *Z. exclamationis* has also been detected in Arizona (Brisley 1925). Schulz (1978) described the sunflower beetle as the predominant leaf feeding species of the sunflower insect complex.

Besides the common annual sunflower, *Helianthus annuus* L., other species of *Helianthus* have been shown to be hosts of sunflower beetle including *H. petiolaris* in Arizona (Brisley 1925) and *H. giganteus* in Manitoba (Criddle 1922). From feeding and survival studies on various *Helianthus* species, *H. debilis*, *H. paradoxus*, *H. angustifolius*, *H. argophyllus*, and *H. mollis* would also be expected to be hosts of sunflower beetle (Rogers and Thompson, 1978). Rogers (1977) described *Franseria tomentusa*, wooly leaf bursage, as a host.

Adult beetles are 6-8 mm long, with a dark brown head and thorax, while each elytron has pale longitudinal stripes alternating with dark ones. Laterally, a dark stripe and a dot are found which resembles an exclamation mark. Eggs are yellow and may change with age to orange. The eggs are 1.5- 2 mm long and are cigar-shaped. They develop into yellow humpbacked larvae in which the fourth and last instar is 10 mm long.

## Biology and Population Dynamics

Sunflower beetles emerge as adults in the spring on various dates, beginning with those on the southern Great Plains. Adults were commonly found on bursage before April, the earliest emergence date of commercial sunflower in Texas (Rogers 1977). In North Dakota Charlet (1991) found the earliest emergence was 20 April and the latest was 27 June. Fifty percent emergence occurred between the 129th and 136th day of the calendar year, or about the 131st day. The calendar date was between 9 and 15 May. Eighty percent of the adults emerged by late May or early June as the first of the commercial sunflower was emerging. The number of degree-days required for first emergence was shown to be 232 in North Dakota, and for 50% emergence, 395 days. A threshold for determination of the degree-days required for emergence

was set at 0° at 5 cm of soil depth (Charlet 1991). In Manitoba, the mean date of first emergence was estimated to be 20 May (Neill 1982) or 235 degree-days when the threshold, later used by Charlet, was also employed. Males and females emerged with equal frequency statistically, although there is a preponderance of males at the earliest dates (Charlet 1991). Neill (1982) showed that males predominated during the earliest emergence, but became equal by 50% emergence.

After mating, Neill (1982) measured a 2.5 day preoviposition period, with oviposition continuing for 60.7 days. Charlet (1996) measured oviposition continuing for 39.4 days. Neill found that female fertility was 30.7 total eggs/female/day, while Charlet found that it was only 2.3. Eggs required 5.6 days of development at 25° C (Neill 1982). Charlet (1996) showed that fecundity apparently varies with environmental or biological conditions. Of those eggs collected on 16 June 1995, 54.7% were shrivelled and did not hatch and 59.3% of the remainder hatched for a total of 26.8% of all eggs. On 29 June only 9% of the eggs were shrivelled, and 66% of the total oviposited eggs hatched. About 73% of the eggs oviposited in the laboratory successfully hatched.

The first larvae in the sunflower fields of North Dakota begin to be seen June 5-10 (Charlet 1992) and the peak appearance of larvae is June 14-20. The last larvae are seen in mid to late August. Neill (1982) has shown the length of the 4 larval instars at 25° C to be:

$L_1$- 4.5 days
$L_2$- 3.2 days
$L_3$- 3.6 days
$L_4$- 4.1 days

The larvae pupate in the soil at the base of the sunflower plant (Charlet, 1992). A non-feeding prepupal period lasts 4.7 days at 25°, and the pupal development 7.2 days (Neill 1982). Adults emerge in late July and early August and feed without any substantial damage to commercial sunflower (Charlet 1992). At this time, the adults are commonly found on the bracts and other parts of the sunflower head and are in reproductive diapause.

Between the first and third weeks of September the adults are no longer detected, having entered the soil for overwintering (Charlet 1992). The insects are found mostly at a depth of less than five centimeters (Neill 1982). The cues triggering diapause in the sunflower beetle probably occur during larval development, since relatively long daylength is part of the signal to diapause. Neill (1982) has shown that temperature is also important in determining of diapause, in addition to the photoperiod. At 15-20° C. and a light regime of 16L:8D diapause is induced in 100% of the adults. At 25° C diapause is induced in only 40-47% of the adults. With a light regime of 15L:9D there was 0% diapause at 22° C.

Insects can be reared in the laboratory on sunflower, and Neill (1982) described the procedure. He used a 15L:9D photoregime at 22° and 70% RH to maintain a non-diapausing colony. It should be noted that maintaining reasonable densities of insects requires frequent moving of larval insects onto new plants, and that host plants need to be completely insect and

pathogen free so that the insects will feed on the plants (Roseland, unpublished). A loss of about 20% of early larval instar insects to unknown causes is typical, and a loss reaching nearly 50% is common for pupating insects.

Economically important damage may occur on newly emerged sunflower around the time of adult emergence in the spring (Charlet 1983, 1996). Adult feeding damage occurs on the leaves at the margins. At low densities sunflower beetles generally avoid feeding on the cotyledons. Zhang (NDSU Entomology Dept., unpublished) has shown that adults will feed on cotyledons if no other choices are given, but larvae will not do so. Adults actively feed during the day. Complete defoliation of seedling sunflower plants is possible by either adults or larvae.

Larvae feed on the leaf surface, both the margins and on sites away from the margins. Charlet noted that larvae may also feed on immature disk flowers (1992). Larvae feed mostly at night, and congregate around structures near the central axis of the plant. Westdal (1975) concluded that larvae cause most of the economic damage, but this finding may be characteristic of Manitoba fields. New generation adults that are in reproductive diapause cause minimal economic damage, although severe shotholing may be seen in mature plants at their late July emergence, which can of course be attributed to earlier damage by both larvae and adults.

The amount of damage attributed to the sunflower beetle may vary widely from year to year. From the records of the North Dakota State Entomologist, in 1982, only 1% of over three million acres needed an aerial application of pesticide for the control of this pest (D.Nelson, unpublished). In 1983-1987 when on average over two million acres were planted yearly, 26 percent of the total acres were treated for sunflower beetle (Nelson 1994). During this five year period, the records showed that about 45% of the acres received aerial application of a pesticide in one of the years. Between 1988 and 1992, less than 10% of the acres were treated for sunflower beetle. By 1993 and 1994, about 48% of the acres treated for any pest were for sunflower beetle infestations (D.Nelson, unpublished and Nelson 1994). An unknown amount of systemic insecticides were also used in those years for early season beetle control.

**Management Strategies**

Larvae were considered to be the most damaging stage of the sunflower beetle on sunflower in Manitoba, based on the report of Westdal (1975). This report indicated that at least 25 or more larvae per plant can lead to complete defoliation and reduce yields as much as 30%. Thus the Province of Manitoba recommended (1979) that chemical controls should be applied when population densities reach more than 10 larvae per plant. Reviewing this recommendation, Charlet (1981) infested caged sunflower at the 4-5 leaf pair stage with up to 20 larvae per plant. He found no decrease in yield, in seeds per head, or in head diameter. However, it may be useful to repeat this work starting with younger plants which may be more susceptible to the consequences of larval pressure.

The effects of various numbers of adult beetles feeding on commercial sunflower have been described in somewhat more detail than have the effects of larval feeding. Charlet (1983)

subjected sunflower in cages to pressure of 2, 4, 6, 8, and 16 beetles/plant. The insects were introduced to the plant at the third leaf pair stage. The results were:

| | |
|---|---|
| 2 adults or more | significant decrease in yield (20%) |
| 4 adults or more | significant decrease in leaf surface area |
| 16 adults | significant decrease in oil percentage in seeds |
| all treatments | significant decrease in number of seeds/head |

Chemical means are at present the only strategy for controlling infestations of the sunflower beetle. The recommendations published by the North Dakota State Cooperative Extension Service for an economic threshold are one to two adults per seedling plant (McBride and Charlet 1985). An economic threshold for larval infestations is attained when 25-30% of the plant is defoliated. Even then, no control is advised unless the larvae are less than 1/4 inch in length and more damage is expected. It is suggested that only 10-15 larvae per plant attacking the upper eight to twelve leaf pairs are sufficient to attain the economic threshold.

The pest scouting program described by the North Dakota Cooperative Extension Service (McBride and Charlet 1985) proposes sampling of 100 plants for larvae or adults at five locations in a field, and recommends against any monitoring within 75 to 100 feet of the field margins. Determination of the percent defoliation should be based upon 100 plant samples at five locations for a total of 500 plants. Obviously such prodigious sampling is needed to provide a reliable estimate of pest status and damage, but pest management advisers often do not use them (personal communication). Perhaps an estimate of reliability based upon smaller samples would assist them in the practical application of the guidelines for this type of scouting recommendation.

**Biological Control.** The investigation of mechanisms to enhance biological control of sunflower beetles should be encouraged. Several insects may be useful to growers as an alternative to chemical controls alone. If biological agents can be surveyed in the field before a chemical treatment decision is made, and the adequacy of certain populations of predator or parasite are known, chemical pesticides may not be required in every situation.

Neill (1982) has provided an extensive list of parasites and predators of the various stages of sunflower beetles. Egg predators in Manitoba have been identified as ladybugs (*Hippodamia*) and soft-winged flower beetles (*Collops*). Predators which feed on both eggs and larvae include *Hippodamia*, and lacewings (*Chrysopa*). Exclusive larval predators include the carabid beetles (*Lebia*), stinkbugs (*Perilliodes*) and the damselbugs (*Nabis*). Adult predators include stinkbugs (*Podisus*) and blackbirds.

The sunflower beetle is attacked by three principal parasites. The tachinid fly *Myiopharus* sp. overwinters as a first instar larva in adult beetles, and emerges in the spring following the death of the host. Two generations per year are reported (Neill 1982) and infestation reaches 1.7% of the diapause destined adults (Charlet 1992). The larval parasite *Doryphorophaga macella* is another tachinid fly parasite that oviposits on the first two instars of the larval host. After pupation of the host the fly exits from the pupa while it is in the soil. The female is very fecund and may lay 285 eggs in her lifespan (Neill 1982). This rate results in up to 70%

parasitization (Neill 1982) or 67% (Charlet 1992). A third major parasite is the egg parasite *Erixestus winnemana*, a Pteromalid wasp. This insect has a 16 day life cycle (Neill 1982) and since it cannot overwinter as a sunflower beetle parasite, it is thought to seek some other chrysomelid host (Charlet 1992). Charlet (1992) found that *Erixestus* attained a parasitization rate of nearly 2% in the field.

**Cultural Control.** Few reports of strategies for the cultural control of sunflower beetle have been published. Some work has begun on the use of planting strategies in which resistant and susceptible plants are interplanted to decrease the total amount of feeding (Grosz and Roseland 1994, and Roseland and Grosz 1996), but so far this work has been accomplished only in cage assays. When modestly resistant plants were interplanted at 25% of the total with susceptibles making up the remainder, feeding was reduced by 20% on the resistant plants (Grosz and Roseland 1994). When resistant plants were planted around the periphery of a block of susceptibles, total feeding was reduced to 65% of that in cages in which a susceptible population surrounds a block of resistant plants. Total feeding in these same cages with a resistant plant border was 75% that of cages with adjacent solid blocks of both plant types (Roseland and Grosz 1996). Perhaps other combinations of plants will provide even greater reductions in feeding. Replication of these results in small field plots will be needed to confirm the usefulness of this stategy.

**Chemical Control**. There are currently seven chemicals registered for sunflower beetle control in the state of North Dakota (six: Glogoza 1996 and Baythroid: Glogoza, personal communication). These include foliar and sytemically applied formulations.

**Plant Resistance.** If the antixenotic properties of some of the non-*Helianthus annuus* sunflower plants can be introduced into commercial hybrids, at least four species would provide substantial resources. Rogers and Thompson (1980) showed that *H. tubersosus, H. salicifolius, H. ciliaris* and *H. paradoxus* cause 90-100% mortality to the larvae reared on them and Rogers and Thompson (1978) showed that another 17 *Helianthus* species likewise caused substantial mortality. These plants may also express a deterrent to oviposition against sunflower beetles, since on the average, no eggs were found on them. Another promising strategy is that of Grosz and Roseland (1994) in which individual phenylpropanoids (as a class, these are frequently deterrents and antibiotics) were quantified in various wild and inbred lines, and then were related to the specific deterrence of each line. This resulted in the identification of one and perhaps a second unknown chemical eluting at specific retention times that correlated well with the deterrence as it was assayed on whole plants (Satter 1996). If this relationship can be demonstrated in other lines, assays for these specific chemicals can be made in additional populations of sunflower. This work may reveal new lines with exceptionally high levels of deterrence towards sunflower beetle.

## Summary and Future Needs

The details of the life history of the sunflower beetle are well investigated. Other details about how the insect is distributed and how feeding affects sunflower yield need more research. We would like to know how the insect distributes itself from the emergence site to the infestation

site, and once arrived, what distribution it assumes within a field. While we presume that most overwintering occurs in the soil beneath the plants on which the insects have developed, we do not know if more distant sites are also used. These facts would be needed to know whether tillage may be a suitable treatment to disrupt the overwintering of adults. A detailed description of how much defoliation can be tolerated before yield declines, both at different growth stages, and in the upper or lower part of the plant, is needed. The sampling strategy recommended by the North Dakota Extension Servise as we previously noted is too lengthy, and a plan needs to be devised which would give a margin of error for differing sampling efforts. Perhaps a sequential sampling plan could also be worked out for the sunflower beetle. An attempt to enhance the parasite populations around planted fields might be attempted, for example, by growing multiple headed plants which display continous flowering. With sunflower hosts in all stages of flower growth perhaps the presence of parasites and predators could be prolonged and thus provide support for a biological control effort. A continuing effort is being made to find resistant lines and to use existing varieties in a management plan. Finally, the consequences of the date of planting on sunflower beetle populations have not been investigated, and this might be a useful adjunct for the management of this insect on sunflower.

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# THE BIOLOGY AND MANAGEMENT OF THE BANDED SUNFLOWER MOTH, *COCHYLIS HOSPES*. PAST, PRESENT, AND FUTURE.

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The USDA has made a comittment to have implemented Integrated Pest Management (IPM) systems on 75% of all U. S. acreage by the year 2000. IPM is a system that combines biocontrol, cultural control and chemical control with careful monitoring of the distribution, population densities, seasonal bionomics and biology of pest insects and their natural enemies. The following presentation is a summary of past and present research on the implementation of IPM of *Cochylis. hospes,* the banded sunflower moth (Lepidoptera: Cochylidae) and future prospects for management of this pest.

**Distribution of the Banded Sunflower Moth:** In a pheromone trap study, Beregovoy (1989) described the distribution of *C. hospes* over a vast area of North America from Manitoba in the north to Santa Clara Canyon, Mexico in the south and to North Carolina and New Jersey in the east. The distribution of *C. hospes* west of a line from the western Dakotas to Texas was sparsely defined although there was one report from an Oregon site. The greatest populations were in the important sunflower growing regions in the Great Plains area particularly the Dakotas and Minnesota and increasing in areas like Colorado and Kansas where sunflower acreage is expanding. There is also a subspecies, *Cochylis arthuri* (Dang, 1984), in the area around Saskatoon, Saskatchewan, Canada and in eastern North Dakota (T. Gross personal communication). World wide there are about 18 species in the genus *Cochylis* known from the Holarctic, Oriental and Neotropical regions (Razowski, 1984).

**Description of the life stages of the banded sunflower moth:** The adult banded sunflower moth is a small moth about 1 cm in length with a namesake band of brown scales across the midsection of the forewings. The eggs are generally oval in shape and initially white in color, but turn yellowish brown as the larva and headcapsule develop. The time needed to hatch and the development of the five instars decreases with increasing temperature, although, mortality

increases sharply above 30°C. The larvae are a yellow-tan color but when the fifth instar ceases to feed it undergoes a series of color changes during wandering behavior prior to diapause.

**Life cycle and seasonal history:** The life cycle of the banded sunflower moth was initially described by Westdal (1949, 1955, 1975) and further refined by Beregovoy and Riemann (1987), Beregovoy, et al. (1989), and by Charlet and Gross (1990). It is a univoltine species in the northern Great Plains. Adult moths begin to emerge from the soil about mid-July. The eggs are laid on the bracts of developing sunflower heads and leaves near the head of the plant. Westdal (1949) indicated that sunflower heads in the pre-flowering bud stage were preferred for oviposition. The eggs hatch in 5 - 8 days and feed on the florets and developing seeds as they progress through five instars. The fifth instar feeds voraciously for about two days and then undergoes color changes and wandering behavior and drops to the ground to overwinter inside a cocoon that it spins in the upper few centimenters of the soil. Banded sunflower moths have a facultative diapause (Barker, 1992). Diapause is induced primarily by temperatures below 20°C. A short photophase has a role in diapause induction but has less influence than temperature in diapause induction of this species. We take advantage of their facultative diapause to rear them continuously in the laboratory.

**Emergence period:** The emergence period for the banded sunflower moth in the northern Great Plains area was described by (Beregovoy and Riemann 1987; Beregovoy et al. 1989; Charlet and Gross, 1990). Emergence occurs in early July but minor differences in emergence date that depend on weather conditions and soil temperatures were detected from year to year at different locations. Beregovoy (1989) indicated that *C. hospes* tended to emerge later in areas outside the eastern ND region. In the far south, we are not sure that emergence is the same as in the north. Beregovoy says the flight period begins in Pharr, Texas about June 20, but then he didn't set up his traps before June 20. Beregovoy (1989) speculated there may be more than one generation but no one has observed that they do. He indicates that emergence of *C. hospes* in Texas and Arkansas was less synchronized than in the Northern Plains and possibly there is more than one generation. Physiologically they are capable of more than one generation and

anywhere the photophase and temperature are right and there is a host plant available this moth could go through a reproductive cycle without diapause.

**Fecundity:** The fecundity, or number of eggs oviposited will depend on the temperature. Table 1 contains ovipostion data from an unpublished study (Barker). Five female and five male moths were collected shortly after emergence and placed in a small cage with a bud stage sunflower head from one of the varieties listed below. The temperature was held at 28°C and the relative humidity was 50%. The total number of eggs laid over a 8 - 10 period were counted. Most of the moths were dead after 8 - 10 days.

**Table 1. Fecundity of female banded sunflower moths.**

| Variety | Total # eggs | eggs\female |
|---|---|---|
| 175730 | 513 | 102.6 |
| Yellow 316 | 506 | 101.2 |
| 251465 | 332 | 66.0 |
| 170408 | 637 | 127.0 |
| 1843 | 283 | 56.6 |
| 170407 | 671 | 134.2 |
| 343799 | 652 | 130.4 |
| 4069 | 703 | 140.6 |
| Maximilliani | 557 | 111.4 |
| 4393 | 279 | 74.2 |
| 913066 | 808 | 161.6 |
| 413034 | 671 | 134.2 |
| 4049 | 785 | 157.0 |
| 3262 | 772 | 154.0 |
| 413034 | 1023 | 204.6 |
| 413119 | 931 | 186.4 |
| 175730 | 1157 | 231.4 |
| Stauffer 1300 | 1508 | 301.6 |
| 894 | 889 | 177.8 |

Ave. = 144.8 ± 13.3

Range 56.6 - 301.6

**Damage and the pest stages involved:** *C. hospes* larvae are the most damaging phase of the life cycle. Beregovoy and Riemann, (1989) indicated that the seed of the sunflower was the chief food source although according to studies by Westdal (1955) the early first and second instars feed on the florets and pollen and by the third instar they penetrate into the seeds eating through the hull and consuming all or part of the seed inside. Mature seeds were eaten fom the inside and the larva exited through a hole usually at the crown of the seed but sometimes the larvae consumed part of the shell. Westdal (1949) reported the damage to vary from 1.7 and 3.5% in some years and in some locations up to 40% of the seed was damaged. Charlet and Gross (1990) found the damage to range from about 8 to 20% of the seed. The number of seeds that were entirely consumed averaged 6 - 7.

**Alternate hosts:** Although important knowledge for IPM, potential alternative hosts other than wild or cultivated sunflower have not been identified for the banded sunflower moth.

**Economic injury level / economic threshold:**

Economic injury level and economic threshold are terms that relate pest numbers with damage cost and management costs and are a deciding factors in determining whether to treat or not to treat. Charlet et al. (1995) and Charlet and Barker (1995) provide details for the calculation of an economic threshold and an economic injury level, respectively for the banded sunflower moth. For the banded sunflower moth, the number of adult moths that might cause economic injury was determined to be 1 adult moth per 56 sunflower plants (Charlet et. al., 1995)

**Pheromones or attractants:**

Components of the female pheromone to attract male banded sunflower moths have been identified as (E)-11-tetradecenyl acetate and (Z)-11-tetradecenyl acetate (Underhill *et al.*1986).

**Sampling /monitoring of banded sunflower moth adults, larvae or eggs:**

**Pheromone Trap Monitoring:** The male attracting pheromone was impregnated in red rubber dispensers and attached to the inside of delta-type traps with an internal sticky surface. The traps were mounted horizontally on bamboo stakes with rubber bands 30 cm above the canopy of sunflower or other vegetation (Beregovoy 1989). The pattern of trap set ups is up to the investigator but a common field method is to set up 5 sites in an X pattern in a field or plot and sample for male adult moths in each corner of the plot and at the center. Some investigators may prefer to set up additional traps or sample sites in the vegetation around the field because banded sunflower moths may tend to congregate in the vegetation around a sunflower plot or field according to Beregovoy (1987).

**Survey and count methods:** Sampling for adults, larvae or eggs may be conducted with survey and count methods following the X pattern of survey sites. Adults may be counted on 25 or 50 plants at each of 5 sites or 2-5 heads may be taken from each site and the number of larvae and eggs determined at the lab by dissecting them from the heads.

**Laboratory rearing:** A method to rear the banded sunflower moth has been developed (Barker, 1988). Although laboratory rearing of the banded sunflower moth is not essential to the development of IPM, year around availability for experimentation facilitates the development of IPM methodology.

**IPM management techniques:** Biological, cultural, and chemical control are the central components of IPM. The first of these, biocontrol is the use of insect predators, parasites, host plant resistance, and diseases to control insect pests.

**Parasites and predators:** Studies to identify the predators and parasites of the banded sunflower moth have been conducted by Charlet (1988), Bergmann and Oseto (1989). Bergmann and Oseto (1989) identified birds as predators of about 8% of banded sunflower

moth larvae. Insects predators and parasites included *Orius tristicolor* (Hemiptera: Anthocoridae), Ground beetles (*Pterostychus lucublandus*), *Glypta* (Ichneumonidae), and *Chelonus* (Braconidae). In all, predation was identified by Bergmann and Oseto (1989) as the factor killing 92.5 and 84.8% of banded sunflower moth pupae in a two year study. Charlet, (1988) identified *Glypta* n. sp. (Ichneumonidae) and *Chelonus phaloniae* (Braconidae) as the primary parasites of the banded sunflower moth in the northern Great Plains The frequency of parasitization varied in different years with *Glypta* predominating in some years and *Chelonus* in other years. *Macrocentrus ancylivorus* (Braconidae) and *Perilampus robertsoni* (Ptermalidae) were also collected but were found in significantly lower numbers than *Glypta* or *Chelonus*. *Bracon mellitor. Mastrus* n. sp. (Ichneumonidae) and *Trathala* sp. were collected in some years in low frequency. Other parasites of the banded sunflower moth include *Chelonus shosphoneanorum* (Braconidae), *Brassus arthurellus* (Braconidae) have been collected in other geographical areas but not in the northern Great Plains.

**Host plant resistance:** Research by Charlet and Brewer (1995) showed that *H. annuus*, from which hybrids are derived, is the most susceptible of six common wild sunflowers from North Dakota to the banded sunflower moth. Banded sunflower moth larvae infested about 25% of the *H. annuus* heads and about 12% of the heads of *H. petiolaris petiolaris, H. maximiliani, H. tuberosus, H. rigidus subrhombiodeus,* and *H. nuttallii rudbergii.* Banded sunflower moth larvae have been collected from *H. floridanus, H. praecox*, and *H. argophyllus* by other workers. It has long been known that wild sunflowers are more resistant to insects than are cultivated sunflower. Sunflower is a native species and insects have had a long co-evolution. A number of papers have been published that reconfirm this. Not a great deal of progress, however, has been made to identify specific sunflower traits, or chemicals that specifically resist feeding, attraction or oviposition of the banded sunflower moth.

Florets have been reported to have larvicidal effects on another moth species (Waiss, et. al., 1977). I could not confirm similar larvicidal effects of florets on banded sunflower moth larvae

but the acetone extractable material from seeds contained component(s) that delayed development and significantly increased mortality (Barker, submitted to Environ. Entomol.)

**Table 2. Effect of sunflower florets and seed on the mortality and development time of banded sunflower moth larvae (mean ± SEM).**

| Diet Treatment | Total Development time (days) | % mortality | n |
|---|---|---|---|
| Unextr. seed | 24.9 ± 0.4 | 44.1 ± 4.0 | 3 |
| Acetone extr. seed | 21.7 ± 0.1 | 19.2 ± 3.1 | 3 |
| 100 g extr. florets | 30.8 ± 0.3 | 11.7 ± 4.3 | 3 |
| 100 g intact florets | 33.1 ± 0.2 | 15.0 ± 3.1 | 3 |

Pollen inhibited banded sunflower moth oviposition on an artificial substrate (Barker, submitted to Great Lakes Entomol.). *C. hospes* gains some reproductive advantage from ovipositing on the bracts of sunflower heads. Pollen may play some role in selection of the oviposition site by discouraging oviposition on advanced stage sunflower heads with open inflorescences. Leaves and bracts on the other hand are quite attractive as an oviposition site and about equally attractive. The leaves and bracts of sunflower contain a combination of contact chemicals, volatiles, moisture and texture that enhanced oviposition by the banded sunflower moth. The active material in pollen was found to be soluble in water. If the deterrent factor in pollen is eventually identified and it can be amplified in the leaves and bracts of the sunflower through plant breeding or genetic engineering it could be useful in discouraging the banded sunflower moth from ovipositing on sunflower.

One of the most promising of all developing control strategies is genetic engineering of sunflower to resist insects with Bt (*Bacillus thuringiensis)* toxin genes. In this case, insect resistance is built into the sunflower seed and the grower does not have to be concerned about tricky manipulations of other biological control and cultural control techniques. This technique

too has its problems and the way is not paved with gold. Currently there is an intensive effort to introduce Bt genes into sunflower. The Bt toxin gene has been sucessfully introduced into a number of crops such as corn, cotton and potatoes and will eventually be introduced into sunflower. Bt is a gram positive soil bacterium. Bt cells produce proteinaceous crystals that are toxic to insects. The genes for the protein may be located on the bacterial chromosome or on plasmids (Gelernter, 1992). Thousands of different types of protein exist, some of which are quite specific for different orders of insect. Bt was discovered around 1901 by the Japanese bacteriologist S. Ishawata (Beegle and Yamamoto, 1992). Its use as an insect control agent was recognized immediately. The crystalline protoxins range in size from 27 to 140 Kilodaltons. The protoxin dissolves and is cleaved by proteases in the alkaline pH of the insect midgut. Electrophysiological and biochemical evidence suggests that the toxins tear holes in the midgut epithelium cells of susceptible insects disturbing osmostic balance causing cell swelling and lysis and gut paralysis. Infected larvae stop feeding almost immediately. Susceptible insects rarely recover to feed and die of starvation.

We were unable to simply order Bt toxin from a chemical company and we had to make our own preparation for experiments on the banded sunflower moth. The material we prepared was very active (Table 3).

**Table 3.Lethal dosages for 10. 50 and 90 % mortality of banded sunflower moth lavae.**

| Instar | LD10 | LD50 | LD90 |
|---|---|---|---|
| 3rds | 0.024 ug/ml | 0.13 ug/ml | 0.71 ug/ml |
| 4th | 0.057 | 0.22 | 0.91 |
| 5th | 0.2 | 0.54 | 1.40 |

The larvae do not drop dead immediately. Feeding does stop abruptly within hours after placement on infected diet. Death seems to occur by starvation over a period of time. Each instar takes longer to succumb than the previous one because each sucessive instar has had a longer time to feed on regular diet and build up energy reserves before transfer to Bt diet. First

instars were dead in just under 5 days while a fifth instar survived for an average of 18 days. In any case, a transgenic plant that produced Bt toxin in quantities of 1 ug/ mg of sunflower tissue may be resistant to the banded sunflower moth.

**Diseases:** Insect viruses have potential as biological insecticides and can be an important aspect of IPM procedures. Viruses are often host specific and less likely to harm non-target organisms and beneficial insects. Pathogenic viruses that infect the Lepidoptera include the Baculoviridae, Poxviridae, Iridoviridae, Parvoviridae, Reoviridae, and Picornaviridae (Adams and Bonami, 1991). We have found a filamentous virus that has pathological effects on banded sunflower moth larvae. Within a few hours after eating contaminated diet, the larvae showed signs of acute pathgenic infection. They became sluggish, ceased feeding, and the tissues of the body quickly disintegrated and turned brown and then became black. We have not yet identified the virus but we intend to investigate the potential of this virus as a control agent. It may be a Baculovirus. Baculoviruses cause lethal disease in insect larvae and have been described from over 600 species of insects including Lepidoptera, Hymenoptera, Diptera and Coleoptera (King and Possee, 1992). If chemical insecticides had not been so successful and cheap in controlling insect pests, the baculoviruses might have played a more prominent role in agriculture. Unmodified baculoviruses have been used sucessfully as insecticides, but suffer from the disadvantage of slow action compared to chemicals. The potential of baculoviruses as an insecticide can be increased, however, because they can be genetically modified because of the presence in the viral genome of the polyhedrin and P10 genes. Both of these genes have powerful promoters and produce copious amounts of protein to construct proteinaceous inclusion bodies that function to encapsulate and protect viral particles and act as a dispersal agent between individuals. Deletion of the polyhedrin or p10 and insertion of a foreign gene has been particularly useful in producing protein products. Some research has been done to modify baculoviruses to make them more suitable as insecticides such as insertion of juvenile hormone esterase which upset the hormonal control of growth an development of the target insect. The Bt gene has also been inserted to produce that protein but it is possible that there are a number of

proteins that like Bt toxin fortuitously have insecticidal properties. The Poxviridae and the Reoviridae also have inclusion bodies and may have potential for similar modifications but these have not been investigated as extensively. The Poxviridae are not associated with insect epizootics although the Reoviridae or cytoplasmic polyhedrosis viruses are disease producing viruses (Adams and Bonami, 1991).

Banded sunflower moth larvae have also been found to be susceptible to *Isaria* a fungal pathogen (Bergmann and Oseto, 1989)

**Cultural control**: Cultural control includes farming practices already associated with crop production including: planting date, tillage, crop spacing or plant population, trap crops, crop sanitation, and crop rotation. Only those practices for which there is data for banded sunflower moth control will be discussed.

Three studies have been conducted showing that delayed planting reduced damage by the banded sunflower moth, (Charlet and Busacca !986, Beregovoy and Riemann 1987, and Oseto et al. 1989). The idea was to grow the crop or at least its most vulnerable stage when the pest was not present. Adult moths become abundant around July 15 and prefer the R2 to R4 stages for oviposition. So to reduce damage, delayed planting might uncouple the most suitable sunflower head stage with peak moth abundance. Damage is greater around the edges of a field. Delaying sunflower planting until late May or early June will help reduce infestation levels of the banded sunflower moth but the approach has problems for two reasons: first, late planting could be conducive to increased seed weevil damage due to differences in timing of the two insect species; and second, growers may not want to risk the crops ability to reach physiological maturity if weather conditions are bad.

**Tillage:** Tillage of crop residue destroys overwintering insects by burying them or bringing them to the surface where they are exposed. Deep fall plowing of sunflower stubble has been found to reduce moth emergence by 80% the following season (Westdal and Barrett, 1955). The study was conducted in Manitoba and indicated that burying the larvae and cocoons injured

them or made it difficult for the emerging moths to reach the soil surface. Deep tillage, however, has a drawback in prairie areas where there are problems of erosion of exposed soils. Weiss *et al.* (1989) showed gang-discing significantly reduced banded sunflower moth larvae by 20-50%.

**Trap cropping:** With this technique, the main crop is protected with a decoy crop around or adjacent to the main crop to intercept pest insects. A specific study using trap cropping to control the banded sunflower moth was not found, however, banded sunflower moths have a tendency to congregate around field margins prior to flowering (Beregovoy, 1987) and potentially this technique could be of value for control of *C. hospes*.

**Chemical control:** As of March 1996, Furadan 4F, Asana XI, Lorsban 4E, Scout X-TRA and Warrior were registered for banded sunflower moth control . All are classified by the EPA as restricted use pesticides. The economic threshold for treatment was about 1 moth per 2 plants (Glogoza, 1996).

**In summary:** Information from research in the northern Great Plains that is currently available for the develoment of IPM for the banded sunflower moth includes the following: 1) the seasonal bionomics and life cycle have been described by three studies. 2) major parasites and rates of parasitization have been described. 3) cultural controls that include deep plowing and gang-disk tillage have been shown to be effective measures. 4) a pheromone to attract male moths has been identified. 5) work has been initiated with viral, bacterial, and fungal pathogens. 6) chemical control is available.

Further work needs to be done to develop methods to mass rear parasites and predators, and to conserve parasites and predators that currently show promise for significant control of the banded sunflower moth. Some hybrids and particularly wild sunflower show resistance to insects but we know very little concerning chemical constituents and physical characteristics of sunflower that are the basis of resistance. Insect resistance introduced into sunflower via

transgenic sunflower plants such as Bt toxin is a very useful development because the control measures already built into the seed. Potential drawbacks of transgenic plants that have to be addressed include the development of resistance to Bt, transfer of genes to wild sunflower, and impact on non-pest endangered species of Lepidoptera. Viral diseases for the control of the banded sunflower moth have not been explored in spite of sucessful programs in other areas. We are initiating work with a filamentous virus that has been found to cause epizootic and lethal infections in our laboratory colony. A male attractant pheromone is available but little use been made of the pheromone to develop control procedures.

We are cooperating, on a limited scale, with the private sector by assaying insecticidal proteins. In this way, we can assist in the discovery of additional insecticidal proteins that can be incorporated into the sunflower plant. We will need to cooperate with a virologist or insect pathologist to identify pathogenic organisms for the control of the banded sunflower moth. We have cooperated by providing adults and thousands of banded sunflower moth eggs to other scientists and graduate students for research and we have shared information on rearing of the banded sunflower moth with other scientists

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